GABRIEL CAIXETA MARTINS

ESTUDOS ECOTOXICOLÓGICOS SOBRE ARSÊNIO EM SOLOS TROPICAIS: AVALIAÇÃO DO VALOR DE PREVENÇÃO DE ARSÊNIO

LAVRAS – MG
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Prof. Dr. Luiz Roberto Guimarães Guilherme
Orientador

Prof. Dr. José Paulo Filipe Afonso de Sousa
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ECOTOXICOLOGICAL STUDIES ON ARSENIC IN TROPICAL SOILS: ASSESSMENT OF THE ARSENIC PREVENTION VALUE

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APROVADA em 26 de Janeiro de 2018.

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2018

À minha esposa Vanessa Ulhoa Santana de Siqueira.

Aos meus pais José Unilson Martins e Cleide Maria Caixeta Martins.

À minha irmã Raquel Caixeta Martins.

Dedico.

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RESUMO

O arsênio é um elemento-traço tóxico aos organismos e a contaminação dos solos por esse elemento é assunto de importância pública. Assim, desenvolver estudos ecotoxicológicos para arsênio, envolvendo solos locais, contribui para o fortalecimento da base de dados utilizada em tomadas de decisões relacionadas a assuntos ambientais. Por isso, objetivou-se com este trabalho selecionar e utilizar, dentre 11 *endpoints*, obtidos em ensaios ecotoxicológicos de crescimentos de plantas, os mais adequados no estudo da toxicidade do As em solos tropicais. Além disso, buscou-se evidenciar o efeito da contaminação por arsênio nos organismos do solo e testar a adequabilidade do valor de prevenção atualmente vigente no Brasil para arsênio quanto a este fim. Para isso, dois solos naturais (Latossolo e Cambissolo) e um artificial (Solo Tropical Artificial – ATS) foram contaminados para se obter um gradiente de concentrações de arsênio (0; 8; 14,5; 26; 46,5; 84; 150; 270 mg de As kg^{-1}) e utilizados nos ensaios com plantas e organismos do solo. Nos ensaios com plantas, foram cultivadas seis culturas (*Zea mays*, *Oryza sativa*, *Sorghum bicolor*, *Phaseolus vulgaris*, *Helianthus annuus* e *Raphanus sativus*) e avaliados o efeito do arsênio sobre a primeira contagem de plantas emergidas, altura, área foliar, diâmetro do caule, germinação total, índice de velocidade de emergência, massa seca, número de folhas, sobrevivência de plantas, índice SPAD e contagem final de plantas emergidas. Esses *endpoints* foram classificados quanto a sensibilidade (valor do EC_{50}) e confiabilidade (amplitude do intervalo de confiança) para a seleção dos mais adequados. Esses parâmetros, juntamente com os teores de arsênio na parte aérea e os fatores de bioconcentração, foram utilizados para evidenciar a toxicidade do arsênio às culturas. Em geral, maior toxicidade foi observada para o solo ATS, seguido do Latossolo e Cambissolo. Dos *endpoints* avaliados, a primeira contagem de plantas emergidas e a massa seca foram considerados os mais adequados, pois apresentam os menores valores de EC_{50} com os menores intervalos de confiança. As espécies *P. vulgaris* e *Z. mays* foram as mais e menos sensíveis à toxicidade do arsênio, respectivamente. Ambas também acumularam os menores teores de arsênio nos tecidos. Nos ensaios com organismos do solo, foram avaliados o efeito do arsênio sobre a reprodução e sobrevivência das espécies *Eisenia andrei*, *Enchytraeus crypticus*, *Folsomia candida* e *Hypoaspis aculeifer*. Não se observou efeito sobre a reprodução e sobrevivência de nenhum dos organismos testes no Cambissolo e, assim como no ensaio com plantas, maior toxicidade do arsênio foi observada para o solo ATS. A espécie menos afetada foi *H. aculeifer* e as mais afetadas foram *E. crypticus* no solo ATS e *F. candida* no Latossolo. Os resultados demonstraram que o valor de prevenção atualmente vigente no Brasil para arsênio é protetivo para solos com maior capacidade de adsorção e pode não ser protetivo para solos com menor capacidade de adsorção de arsênio.

Palavras-chave: Ecotoxicidade. Fitotoxicidade. Arsenato.

ABSTRACT

Arsenic (As) is a trace element toxic to most living organisms and soil contamination with As is a major threat to soils and human health. Thus, developing ecotoxicological studies for As not only at global but also at local scale contributes to develop a more robust database to be used in decision-making actions related to environmental issues. Therefore, the objective of this work was to select and to use the most adequate endpoints - which were obtained in plant growth ecotoxicological assays -, in the study of the toxicity of As in tropical soils. In addition, the aim was to evaluate the effect of As contamination on soil organisms and to test the adequacy of the current soil screening value (i.e., prevention value) in Brazil for As. For this purpose, two natural soils (Latosol and Cambisol) and one artificial tropical soil (ATS) were spiked to obtain a gradient of increasing As concentrations (0; 8; 14.5; 26; 46.5; 84; 150; 270 mg of As kg^{-1}) and used in trials with plants and soil organisms. For the plant trials, six plant species (*Zea mays*, *Oryza sativa*, *Sorghum bicolor*, *Phaseolus vulgaris*, *Helianthus annuus* and *Raphanus sativus*) were grown and the effect of As on the early germination count, plant height, relative leaf area, stem diameter, total germination, germination speed index, dry mass, number of completely expanded leaves, plant survival, soil plant analysis development chlorophyll level, and the final germination count were assessed. These endpoints were ranked for sensitivity (EC_{50} value) and reliability (range of confidence interval) in order to select the most appropriate ones. These parameters, together with As contents in the shoot and the bioconcentration factors, were used to show the toxicity of As on the studied plants. In general, higher toxicity was observed for the ATS, followed by the Latosol and the Cambisol. From the evaluated endpoints, the early germination count and the dry mass were considered the most adequate since they present the lowest EC_{50} values with the lowest confidence intervals. The species *P. vulgaris* and *Z. mays* were the most and least sensitive to As toxicity, respectively. Both species accumulated the lowest levels of As in the tissues. For the evaluated soil organisms, i.e., *Eisenia andrei*, *Enchytraeus crypticus*, *Folsomia candida* and *Hypoaspis aculeifer*, no effect was observed on the reproduction and survival of any of the organisms tested in the Cambisol. Just as in the plant trial, higher arsenic toxicity was observed for the ATS. The species least affected was *H. aculeifer* and the most affected were *E. crypticus* in ATS and *F. candida* in the Latosol. Our findings demonstrated that the current prevention value in Brazil for arsenic is protective for soils with high adsorption capacity and probably not for soil with lower arsenic adsorption capacity.

Keywords: Ecotoxicity. Phytotoxicity. Arsenate.

SUMÁRIO

PRIMEIRA PARTE

1 INTRODUÇÃO GERAL

O arsênio (As) é um metaloide tóxico que ocorre naturalmente no solo e seus teores devem ser monitorados para evitar que cheguem a níveis deletérios aos seres humanos, plantas e organismos do solo. Além dos efeitos tóxicos, o arsênio é também um carcinogênico humano, colocando-o em uma posição de destaque.

Muito tem se falado sobre a exposição dos seres humanos na América Latina ao arsênio via ingestão de águas (superficiais ou subterrâneas) e alimentos contaminados (BUNDSCHUH et al., 2012a, 2012b; CIMINELLI et al., 2017), mas menor atenção tem sido dada aos solos contaminados, principalmente em relação à predição dos efeitos sobre a funcionalidade do solo. Contudo, apesar de ser amplamente estudado, não há consenso sobre as concentrações que são ambientalmente seguras. Isso abre espaço para a investigação de sua concentração no solo, água, ar, sedimentos e sua relação com a toxicidade sobre os organismos, bioacumulação, transferência para cadeia alimentar, segurança alimentar e riscos à saúde humana.

Em relação ao solo, sabe-se que esse é uma matriz complexa, composta por diferentes fases (sólida, líquida, gasosa e viva) que interagem entre si. As inúmeras possibilidades de composição e interações dessas fases condicionam que um mesmo teor total de um contaminante em diferentes solos não apresente toxicidade similar. Estudar a toxicidade de um contaminante em diferentes solos favorece a compreensão de suas interações com os constituintes dos solos e auxilia na predição de seus efeitos para outras situações, tendo em vista a sua disponibilidade. Isso é especialmente importante para arsênio, pois se sabe que a disponibilidade desse elemento é influenciada pelos óxidos de ferro, os quais são abundantes em algumas classes de solos brasileiras. Desta forma, em solos com maiores teores de óxidos de ferro, espera-se maior adsorção de arsênio e, consequentemente, menor disponibilidade e toxicidade aos organismos.

Os teores disponíveis representam a fração do teor total com potencial para causar efeitos adversos, sua utilização para estimar concentrações de efeito tóxico pode gerar valores mais realistas. Além disso, espera-se que sua utilização também reduza as diferenças entre os teores que causam toxicidade em solos com características contrastantes.

No Brasil, a Resolução CONAMA 420 de 2009 estabeleceu os valores orientadores da qualidade do solo, os quais são utilizados na identificação e gestão de áreas contaminadas. Dentre esses valores orientadores, cita-se o valor de prevenção. Esse valor marca a concentração limite de um determinado contaminante, no qual as principais funções do solo são mantidas como, por exemplo, a sua capacidade de sustentar a vida. Assim, trata-se de uma concentração que é ecologicamente relevante, pois deve garantir, no mínimo, que os organismos nasçam, cresçam, sobrevivam e reproduzam. Esses parâmetros podem ser medidos em ensaios ecotoxicológicos envolvendo plantas e organismos do solo.

As plantas são susceptíveis à contaminação por arsênio, contudo, é amplamente conhecido que a sensibilidade não é igual entre as espécies. Além disso, para uma mesma espécie, os sintomas de toxicidade podem não se manifestar com a mesma intensidade entre os diferentes parâmetros de germinação e crescimento. Assim, torna-se necessário identificar quais são os parâmetros mais adequados para serem utilizados em avaliações ecotoxicológicas e quais são as espécies mais susceptíveis à contaminação. Em relação aos organismos do solo, assim como nas plantas, diferentes espécies também apresentam distintas sensibilidades, o que também torna necessário evidenciar o efeito tóxico em diferentes espécies.

Nesse contexto, este trabalho de tese buscou selecionar as variáveis mais adequadas para estudos ecotoxicológicos sobre arsênio e evidenciar a toxicidade do As a diferentes espécies de plantas e organismos do solo, após exposição a três solos tropicais artificialmente contaminados. Os solos utilizados neste trabalho apresentam características contrastantes entre si. Isso foi feito de modo a criar um gradiente de disponibilidade e demonstrar que os teores totais considerados seguros para um determinado solo não devem ser generalizados, uma vez que a toxicidade está diretamente relacionada aos teores disponíveis do contaminante em questão. Dessa forma, enfatizamos que o teor total não é o parâmetro mais adequado para avaliar a toxicidade aos organismos.

Este trabalho de tese foi dividido em duas partes:

a) referencial teórico.

b) artigos:

- artigo 1 e 2 (Experimentos com plantas). Objetivo: selecionar os *endpoints* mais indicados para serem usados em estudos ecotoxicológicos com As, bem como para estudar mais detalhadamente a toxicidade do As em seis culturas de interesse agrícola. Além desse objetivo, avaliou-se a capacidade do valor de prevenção em

proteger as plantas. Espera-se que os resultados gerados possam contribuir para a derivação de valores orientadores da qualidade do solo quanto à presença do As.

- artigo 3 (Experimentos com organismos do solo). Objetivo: estudar os efeitos do As sobre quatro organismos do solo e verificar a adequabilidade do atual valor de prevenção para As (um valor orientador da qualidade do solo adotado no Brasil) em proteger os organismos do solo.

A principal justificativa para o desenvolvimento desses estudos consiste na necessidade de se criar uma base de dados ecotoxicológicas, utilizando solos tropicais, que possa contribuir para a validação e derivação de valores orientadores da qualidade do solo. Isso é necessário, pois é sabido que a ecotoxicologia dos solos é uma área ainda em crescimento no Brasil e que, muitas vezes, devido à ausência de dados nacionais, utilizam-se resultados obtidos em outros países para subsidiar tomadas de decisões envolvendo assuntos ambientais.

2 REVISÃO DE LITERATURA

2.1 Arsênio no solo

O As é um metaloide que tem despertado o interesse de diversos pesquisadores ao longo dos anos, não somente pelos seus efeitos deletérios sobre a saúde humana (BUNDSCHUH et al., 2012b), mas também por ser tóxico aos animais e plantas. De forma natural ou antropogênica, o As pode acumular-se no solo e ser encontrado formando espécies inorgânicas (arsenato e arsenito) e, ou, orgânicas (CULLEN; REIMER, 1989; SMITH; NAIDU; ALSTON, 1998; KABATA-PENDIAS; MUKHERJEE, 2007; KABATA-PENDIAS, 2011; FINNEGAN; CHEN, 2012). Seu estado de oxidação varia entre -3, 0, +3 e +5 (KABATA-PENDIAS, 2011). Nos solos, em ambiente de oxidação, o As^{+5} (arsenato) é a espécie predominante, diferentemente das condições redutoras onde o As^{+3} (arsenito) passa a ser dominante (MEHARG; HARTLEY-WHITAKER, 2002; LIU et al., 2005; GARG; SINGLA, 2011; FINNEGAN; CHEN, 2012). Dessas espécies de arsênio, o arsenito é considerado mais tóxico do que arsenato (VENTURA-LIMA; BOGO; MONSERRAT, 2011; FINNEGAN; CHEN, 2012; YOON; LEE; AN, 2015).

Os teores totais de As nos solos são variáveis em função do material de origem, dos processos de formação do solo e das adições antropogênicas (ADRIANO, 2001; DE SOUZA et al., 2016). O teor médio global de As no solo é 6,83 mg kg^{-1} (KABATA-PENDIAS, 2011). O teor médio de arsênio 5,2 mg kg^{-1} foi encontrado em Latossolos brasileiros (CAMPOS et al., 2007) e em solos do bioma cerrado na região leste de Goiás, Triângulo Mineiro e Nordeste de Minas Gerais foram encontrados os teores de 3,29; 2,18 e 0,62 mg kg^{-1}, respectivamente (CAMPOS et al., 2013).

Teores de arsênio no solo menores que 10 mg kg^{-1} são relatados como naturais (ADRIANO, 2001; CAMPOS et al., 2007, 2013; BUNDSCHUH et al., 2012b), por exemplo, no Estado de Minas Gerais (COPAM, 2011), Estados do Espírito Santo (PAYE et al., 2010) e Estado do Mato Grosso (PIERANGELI et al., 2015) o teor total de As de 8, 12,8 e 2,6 mg kg^{-1} foram associados ao valor de referência de qualidade do solo, ou seja, um teor de *background*. Contudo, deve-se ressaltar que teores maiores são encontrados naturalmente nos solos, como exemplo, 31,7 ± 12,6 mg kg^{-1} para um Latossolo Vermelho no Estado do Paraná (CAMPOS et al., 2007). Isso ocorre quando os solos são formados a partir de matérias de origem ricos nesse elemento (ADRIANO, 2001; PELICA et al., 2018). Por exemplo, na região do Quadrilátero

Ferrífero no Estado de Minas Gerais, no município de Nova Lima, há minérios que podem conter de 0,8 a 8% de arsenopirita (mineral que contém arsênio) (MATSCHULLAT et al., 2000), e isso contribui para que os solos dessa região apresentem maiores teores de arsênio.

Em Minas Gerais, a Fundação Estadual do Meio Ambiente disponibiliza uma lista das áreas contaminadas e reabilitadas no Estado. Na lista do ano de 2017 (http://www.feam.br/servicos-feam/577-gestao-de-areas-contaminadas), foram listadas 32 áreas gerenciadas em decorrência da presença de arsênio no solo. Dessas áreas, 15 estão sob intervenção, 8 sob investigação, 4 são áreas reabilitadas e 3 estão sob monitoramento. Os municípios onde essas áreas se encontram são: Araxá, Betim, Confins, Conselheiro Lafaiete, Contagem, Governador Valadares, Ibirité, Itatiaiuçu, Nova Lima, Ouro Preto, Paracatu, Pedro Leopoldo e Três Marias, sendo as atividades associadas à contaminação os Aeroportos, atividades minerárias e metalúrgicas, deposição de resíduos sólidos urbanos, indústria química e petroquímica (FEAM, 2017). Destaca-se que, no processo de gerenciamento das áreas contaminadas, faz-se uso dos valores orientadores da qualidade do solo, os quais são valorosos instrumentos aos órgãos ambientais.

A toxicidade do As é dependente da concentração desse elemento na solução do solo, ou seja, de sua disponibilidade, a qual é controlada pelos processos de sorção (ROMERO-FREIRE et al., 2014; KADER et al., 2016; LAMB et al., 2016). A disponibilidade é influenciada pelos atributos químicos, físicos e mineralógicos do solo, pelas propriedades do solo rizosférico e condições ambientais (FAROOQ et al., 2016). Dentre os atributos do solo que podem influenciar a disponibilidade, citam-se o pH, potencial redox (Eh), conteúdo de carbono orgânico, textura, óxidos de Fe, Al e Mn, enxofre, fósforo (GULZ; GUPTA; SCHULIN, 2005; WANG et al., 2015; OTERO et al., 2016), carbonato de cálcio, área superficial específica e capacidade de troca de cátions (CTC) (ROMERO-FREIRE et al., 2014).

Contudo, os principais atributos do solo conhecidos por influenciar a disponibilidade de As são o conteúdo de óxidos/hidróxidos de Fe, o pH (KADER et al., 2016) e o potencial redox (Eh) (SHRIVASTAVA et al., 2017). Um maior teor de argila e conteúdo de minerais de ferro contribuem para reduzir a disponibilidade de As (GULZ; GUPTA; SCHULIN, 2005; OTERO et al., 2016). Melo et al. (2012) observaram correlação negativa entre os teores de As nos tecidos vegetais e o teor de argila, Fe_2O_3 e Al_2O_3 presente no solo. Isso ocorre devido à elevada afinidade do As pelos compostos de ferro (ADRIANO, 2001; ZHAO et al., 2009; GARG; SINGLA, 2011; WANG et al., 2015), os quais são descritos como um dos principais

componentes responsáveis por imobilizar As no solo (ROMERO-FREIRE et al., 2014; NGO et al., 2016). Nesse contexto, Drličková et al. (2013) cultivaram plantas de milho em dois substratos contaminados por As e observaram que o solo mais rico em óxidos/hidróxidos de ferro, mesmo possuindo maior teor total de As, proporcionou melhores condições para o desenvolvimento das plantas, devido ao menor teor fitodisponível de As.

Diferentemente de alguns elementos-traço catiônicos (e.g. Cd, Zn, Pb), o aumento no pH pode elevar a disponibilidade do As no solo (ROMERO-FREIRE et al., 2014; ALVES et al., 2016). Considerando-se o efeito do pH, a adsorção de arsenato nos óxidos de Fe e Al é maior em pH ácidos e reduz-se com o aumento do pH, enquanto em pH alcalinos, os carbonatos e óxidos de cálcio passam a desempenhar um importante papel na sorção do As nos constituintes do solo (ADRIANO, 2001; GULZ; GUPTA; SCHULIN, 2005). Além disso, em pH elevados há maior atividade dos íons OH^-, que competem com o As pelos sítios de adsorção na superfície mineral, consequentemente, permitindo uma maior disponibilidade de As (KADER et al., 2016).

Em condições redutoras, o As^{+5} pode ser convertido a As^{+3}. Assim, em ambientes inundados (condições redutoras), ambos os fatores redução do As e dissolução dos óxidos de ferro levam à mobilização do As (FAROOQ et al., 2016). Nesse contexto, materiais orgânicos serviriam de substrato para os organismos, e na sua degradação, os organismos utilizariam outros aceptores de elétrons, como o Fe, reduzindo-os e mobilizando o As adsorvido em sua superfície (ISLAM et al., 2016). Além disso, a própria decomposição da matéria orgânica contribuiria para a liberação do As adsorvido em seus grupos funcionais (FAROOQ et al., 2016). Contudo, deve-se ressaltar que, dependendo das condições pH-Eh do solo, comunidade de bactérias, conteúdo e qualidade de matéria orgânica, os oxihidróxidos de Fe podem permanecer estáveis, mesmo sob inundação (OTERO et al., 2016).

2.2 Efeito do arsênio sobre as plantas

O arsenato (As^{+5}) é um análogo do fosfato e entra nos tecidos radiculares através dos transportadores de fósforo, enquanto o arsenito (As^{+3}) é absorvido via transportadores de silício (ZHAO et al., 2009; SHRI et al., 2009; PANDA; UPADHYAY; NATH, 2010; FINNEGAN; CHEN, 2012; DRLIČKOVÁ et al., 2013; SOUZA et al., 2015; KUMAR et al., 2015; WANG et al., 2015; FAROOQ et al., 2016; WU et al., 2016).

Nos tecidos das plantas, o As^{+5} é convertido a As^{+3} pela ação da enzima As redutase (SOUZA et al., 2015; FAROOQ et al., 2016). Nessa conversão há a formação de espécies reativas de oxigênio (ERO) (PANDA; UPADHYAY; NATH, 2010; TALUKDAR, 2013; FAROOQ et al., 2016). Se o aumento na concentração das ERO for superior à capacidade das plantas em eliminá-las, haverá intoxicação celular e danos oxidativos (LI et al., 2007). Os danos oxidativos (peroxidação das membranas celulares) podem ser indicados pelo aumento no conteúdo de malondialdeído, conforme evidenciado para plantas de *Triticum aestivum* (LI et al., 2007) e *Phaseolus aureus* (KAUR et al., 2012) expostas à contaminação por As.

Além dos danos oxidativos (SHRI et al., 2009), o arsenato interfere no metabolismo do P (SOUZA et al., 2015) e causa distúrbios celulares ao competir/substituir o P em moléculas de ATP e em reações bioquímicas (PANDA; UPADHYAY; NATH, 2010; GARG; SINGLA, 2011; FINNEGAN; CHEN, 2012; YADAV et al., 2014). Esses modos de ação, dentre outros, foram apresentados por Souza et al. (2015) como sendo um dos causadores dos efeitos do As sobre o desenvolvimento de plantas de arroz.

O As nas folhas pode danificar os cloroplastos e reduzir a taxa de fotossíntese (CAPORALE et al., 2013). Reduções na taxa fotossintética também podem estar associadas aos efeitos sobre o ciclo de Calvin e limitações estomáticas (PANDA; UPADHYAY; NATH, 2010). Alguns autores também atribuem o menor crescimento das plantas expostas ao As à redução no conteúdo de clorofila, devido a ela estar associada à biossíntese de carboidratos durante a fotossíntese (YADAV et al., 2014). Redução no conteúdo de clorofila foi observada para plantas de *Phaseolus vulgaris* (TALUKDAR, 2013), *T. aestivum* (LI et al., 2007) e *Pistia stratiotes* (FARNESE et al., 2014) expostas à contaminação por As.

Além da ação sobre o metabolismo do carbono e fósforo, alguns autores também destacam os efeitos no metabolismo do nitrogênio, devido à interferência no suprimento de nitrogênio inorgânico e sobre a sua via de assimilação (FINNEGAN; CHEN, 2012).

A redução no crescimento da parte aérea também tem forte correlação com a redução do crescimento radicular (LIU et al., 2005). Em geral, as raízes são mais afetadas que a parte aérea, pois são o primeiro ponto de contato com o contaminante (SHRI et al., 2009). O As pode causar efeitos negativos sobre o desenvolvimento e anatomia radicular (YADAV et al., 2014), inibir a proliferação, alongamento radicular (FINNEGAN; CHEN, 2012; YOON; LEE; AN, 2015; DE FREITAS-SILVA et al., 2016) e pode causar alterações mitóticas nas raízes (DE FREITAS-SILVA et al., 2016). Segundo Kaur et al. (2012), o efeito sobre o desenvolvimento das radículas seria também devido à menor disponibilidade de sacarose em

consequência do aumento na atividade da invertase ácida. Esses mesmos autores verificaram a perda da integridade da membrana das células de raízes de *P. aureus* quando expostas ao As. Os efeitos negativos sobre o sistema radicular[1], consequentemente, levam a uma menor aquisição/transporte de nutrientes (SHRI et al., 2009), o que também contribui para os efeitos deletérios sobre as plantas.

2.2.1 Sintomas de toxicidade na parte aérea das plantas

A toxicidade do As é variável entre as espécies (KAPUSTKA et al., 1995; KAUR et al., 2012), portanto, os sintomas de toxicidade para duas espécies de plantas podem não ocorrer para um mesmo teor de As. Em geral, manifestam-se os seguintes sintomas de toxicidade em plantas, os quais podem ocorrer isolados ou combinados: aparecimento de manchas cloróticas e necróticas nas folhas (GULZ; GUPTA; SCHULIN, 2005; GUIMARÃES et al., 2016), redução na área foliar, murcha, redução na fotossíntese (LIU et al., 2005), senescência de folhas, redução na produção de biomassa (CAPORALE et al., 2013), redução no crescimento (GULZ; GUPTA; SCHULIN, 2005; YADAV et al., 2014; KADER et al., 2016) e na produtividade (SHRI et al., 2009).

2.2.2 Mecanismos de desintoxicação e proteção

Um dos mecanismos de desintoxicação do As em plantas é a complexação do As^{+3} nos grupos tiol (-SH) presentes em glutationas e fitoquelatinas (MEHARG; HARTLEY-WHITAKER, 2002; RAAB et al., 2005, 2007; SMITH; KOCH; REIMER, 2008; ZHAO et al., 2009; SHRI et al., 2009; SOUZA et al., 2015), bem como sua posterior compartimentalização no interior do vacúolo (SHRI et al., 2009; FAROOQ et al., 2016). O As^{+5} não tem afinidade por grupos tiol, portanto, primeiro é necessário que este seja reduzido a As^{+3} para posterior complexação (PANDA; UPADHYAY; NATH, 2010). Assim, possivelmente, plantas que tenham maior atividade da enzima arsênio redutase sejam mais

[1] Os argumentos relacionados ao sistema radicular sugerem a necessidade de se avaliar *endpoints* tais como o teor de arsênio nas raízes, biomassa e comprimento de raízes. Neste estudo, foram realizadas diversas tentativas de se medirem esses *endpoints*. Contudo, para as maiores doses testadas, não foi possível realizar essas medições de forma confiável. Isso porque, nesses tratamentos, as raízes apresentavam-se muito finas, não permitindo desta forma a sua manipulação. Para determinar os teores de arsênio nas raízes, seria necessário realizar uma pré-lavagem para remoção do solo aderido à superfície radicular. Isso também não foi possível, pelo mesmo motivo acima apresentado. Portanto, esses *endpoints* não foram considerados neste trabalho de tese.

eficientes em desintoxicar o As (PANDA; UPADHYAY; NATH, 2010). Além disso, algumas espécies de plantas também podem suprimir os transportadores de fosfato como uma estratégia para restringir a absorção de As pelas raízes (MEHARG; HARTLEY-WHITAKER, 2002), reduzindo também o fator de translocação, através da redução da solução do xilema (NGO et al., 2016). Há ainda a estratégia de limitar a absorção de As pelas raízes através do espessamento epidermal e também a formação de cristais dentro das células (TALUKDAR, 2013), bem como o aumento da concentração de silício nas folhas, o qual pode levar à sua coprecipitação com elementos tóxicos (SILVA et al., 2015). Destaca-se também a capacidade das plantas de arroz oxidar As^{+3} na zona radicular, bem como o desenvolvimento de placas de ferro nas raízes que atuariam como barreiras adsortivas a entrada de As (SOUZA et al., 2015; AMARAL et al., 2017).

Em relação às condições de manejo de solo, a toxicidade de As pode ser amenizada com o incremento na nutrição de fósforo (CAPORALE et al., 2013). Contudo, devido a similaridade química, arsenato e fosfato competem pelos mesmos sítios de adsorção (MEHARG; HARTLEY-WHITAKER, 2002; GARG; SINGLA, 2011; FINNEGAN; CHEN, 2012) e a aplicação de altas doses de P em solos contaminados pode levar a um aumento do As em solução devido ao efeito de adsorção competitiva (SELIM, 2012).

A fertilização com silício pode reduzir a absorção e os danos sobre o aparato fotossintético de plantas expostas ao As (ISLAM et al., 2016). O silício, além de ser um elemento benéfico para a cultura do arroz, pode contribuir para a redução na absorção de arsenito em virtude da competição pelos mesmos transportadores (WANG et al., 2015; WU et al., 2016). De forma semelhante à interação que ocorre entre o fosfato e o arsenato, o Si pode competir pelos mesmos sítios de adsorção do arsenito, o que consequentemente pode aumentar sua concentração na solução do solo (WU et al., 2016).

O enxofre é um importante nutriente associado aos processos de desintoxicação de As. Assim, o correto status nutricional do enxofre não seria um impedimento à formação de compostos ricos em tiol (-SH), importantes na complexação e sequestro do As (ISLAM et al., 2016).

Além do manejo nutricional, o correto uso da água pode reduzir a toxicidade do As, pois é sabido que condições de anaerobiose contínua levam a um aumento da disponibilidade de As quando comparados a uma condição intermitente de anaerobiose (SOUZA et al., 2015; WANG et al., 2015).

2.3 Invertebrados do solo

A fauna do solo está envolvida em diversas funções ambientais como a decomposição da matéria orgânica, ciclagem de nutrientes, estruturação do solo, regulação parcial da atividade microbiana, dentre outros (CORTET et al., 1999). Devido à sua importância essencial, existem bioensaios laboratoriais padronizados para avaliar os efeitos de contaminantes sobre a reprodução e sobrevivência de organismos do solo, como ácaros (OECD, 2016a), colêmbolos (ISO, 1999; OECD, 2016b), enquitreídeos (ISO, 2003; OECD, 2016c) e minhocas (ISO, 1998; OECD, 2016d).

Ácaros e colêmbolos estão entre os artrópodes mais abundantes e diversos do solo (CORTET et al., 1999; CHRISTIANSEN; BELLINGER; JANSSENS, 2009; OCONNOR, 2009; MOREIRA et al., 2013). Esses organismos são importantes agentes dispersores de bactérias e fungos decompositores da matéria orgânica no solo, contribuem na ciclagem de nutrientes, na decomposição da matéria orgânica, na estruturação do solo e no equilíbrio ecológico (pois podem ser predadores e/ou presas) (CHRISTIANSEN; BELLINGER; JANSSENS, 2009; OCONNOR, 2009; MOREIRA; HUISING; BIGNELL, 2010; MOREIRA et al., 2013; ZHU et al., 2016). Os ácaros possuem alta diversidade, podendo ser encontrados organismos em diferentes grupos tróficos como fungívoros, bacteriófagos, decompositores, herbívoros, predadores e parasitas (OCONNOR, 2009; HUGUIER et al., 2015). A espécie *Hypoaspis aculeifer* (espécie padrão em testes laboratoriais) é um predador generalista, de fácil reprodução e cultura, amplamente distribuída e de representatividade ecológica (SMIT; MOSER; RÖMBKE, 2012; OWOJORI; WASZAK; ROEMBKE, 2014; HUGUIER et al., 2015; MADANI et al., 2015). Os ácaros são considerados, em geral, organismos mais resistentes à contaminação antropogênica que outras espécies de invertebrados terrestres (HUGUIER et al., 2015). Contudo, merece destaque que a espécie *H. aculeifer* é a única espécie de invertebrado predador que possui um protocolo padronizado (HUGUIER et al., 2015). Os colêmbolos são primitivos hexápodes encontrados principalmente nos horizontes superficiais do solo. A espécie *Folsomia candida* (também espécie padrão em testes laboratoriais) possui tamanho pequeno (até 2 mm), não possui asas e olhos, e se reproduz por partenogênese (CROUAU; CAZES, 2005; CROUAU; MOÏA, 2006; CROUAU; TCHIAM, 2006; CROUAU; PINELLI, 2008). Ambas as espécies são consideradas de corpo duro, pois possuem exoesqueleto.

Os enquitreídeos e minhocas são oligoquetas, possuem corpo mole e desempenham um importante papel na decomposição da matéria orgânica e na ciclagem de nutrientes (CORTET et al., 1999; CASTRO-FERREIRA et al., 2012; GONZÁLEZ-ALCARAZ; VAN GESTEL, 2016). Além disso, também contribuem para a formação de galerias no solo, as quais contribuem para uma melhor distribuição da água, ar e matéria orgânica (MOREIRA; HUISING; BIGNELL, 2010). A espécie *Enchytraeus crypticus* (outra espécie padrão em testes laboratoriais) é tolerante a uma grande variabilidade das propriedades do solo como pH (4,4-8,2), teor de argila (1-29%) e conteúdo de matéria orgânica (1-42%) (KUPERMAN et al., 2006). As minhocas estão entre os principais componentes da biomassa do solo, representando uma fonte de alimento na cadeia alimentar, além de desempenhar importante papel na decomposição da matéria orgânica, na ecologia do solo, equilíbrio biológico e estruturação do solo (PEIJNENBURG et al., 1999; LANGDON et al., 2003; LEE et al., 2013; SIVAKUMAR, 2015).

2.3.1 Vias de exposição dos organismos do solo aos contaminantes

Os organismos do solo podem apresentar diferentes rotas de absorção dos contaminantes (PEIJNENBURG et al., 1999). Dentre as fontes de contaminação no solo, pode-se citar o contato com as partículas e solução do solo, ingestão de alimento/partículas e a inalação do ar presente nos poros (SANTORUFO; VAN GESTEL; MAISTO, 2012; PEIJNENBURG et al., 2012). Em relação às vias de exposição, dois grupos podem ser separados: os organismos de corpo mole e os de corpo duro. Os organismos de corpo duro apresentam órgãos especializados para absorção de água/solução do solo contaminado ou partes permeáveis ao contaminante, enquanto que nos de corpo mole a pele é uma das principais vias de exposição (PEIJNENBURG et al., 2012), pois está em constante contato com o contaminante (LANGDON et al., 2003; WATTS et al., 2008). Além disso, deve-se considerar a absorção por alimento para ambos os grupos (VIJVER et al., 2004; PEIJNENBURG et al., 2012). Quando há ingestão de partículas de solo contaminadas, os metais podem ser liberados e assimilados pelo trato digestivo (LEDUC; WHALEN; SUNAHARA, 2008).

2.3.2 Efeito do arsênio sobre os organismos do solo

Alves et al. (2016) estudaram os efeitos do arsênio sobre as espécies *Eisenia andrei* e *Folsomia candida* em um Latossolo e um solo artificial tropical. Os solos testados por esses autores foram incubados durante 30 dias (com umidade de aproximadamente 60% da capacidade máxima de retenção de água) com as seguintes doses de arsênio: 1, 5, 15, 45 e 135 mg kg^{-1}. Esses autores não observaram efeitos sobre a reprodução desses organismos quanto expostos ao Latossolo contaminado por arsênio. Contudo, para solo artificial, observaram redução na reprodução *E. andrei* e *F. candida* a partir da dose 45 e 1 mg kg^{-1}, respectivamente. Esses resultados estão em consonância aos de Romero-Freire et al. (2015). Esses autores investigaram a toxicidade em *E. andrei* de sete solos naturais, representativos da Espanha, contaminados artificialmente com arsênio (0, 50, 100, 300, 600 mg kg^{-1}, incubados por 4 semanas). Esses autores observaram correlação negativa entre os teores de arsênio (total e solúvel em água) e a produção de juvenis. Os valores de EC_{50} para esse *endpoint* variou amplamente entre os solos (56 a 151 mg kg^{-1}). Os resultados desse trabalho evidenciam que os efeitos tóxicos do arsênio sobre *E. andrei* têm forte influência dos teores disponíveis e concentração interna nos organismos. Desta forma, os atributos do solo devem ser considerados nas avaliações de toxicidade, pois podem controlar a disponibilidade e, consequentemente, influenciar a toxicidade.

Em outro estudo, Alves e Rietzler (2015) avaliaram, através de ensaios agudos e crónicos, a toxicidade do arsênio a espécie *E. andrei* quando exposta a 5 solos do entorno de áreas sob influência da mineração de ouro no quadrilátero ferrífero, Estado de Minas Gerais e em um solo artificial tropical. Os solos utilizados M0 (controle de M1), M1, P0 (controle de P1 e P2), P1 e P2 apresentaram teor total de arsênio de 13,2, 489, 82,3, < limite de detecção e 1.329 mg kg^{-1}, respectivamente. Esses autores não observaram efeitos agudos, mortalidade e nem efeitos sobre a biomassa dos organismos. Contudo, a reprodução foi reduzida em 80 e 57% para os solos M1 e P2, respectivamente. Esse estudo, juntamente com os de Alves et al. (2016) e Romero-Freire et al. (2015) evidenciaram que as diferentes toxicidades observadas entre os solos estão relacionadas à disponibilidade do arsênio, reforçando que os teores totais não devem ser exclusivamente utilizados na avaliação de risco de áreas contaminadas.

A importância de se conhecer a disponibilidade do arsênio em estudos ecotoxicológicos também foi confirmada por Chapman et al. (2016). Esses autores atribuíram à mortalidade das minhocas a fração extraível e verificaram que pequenas variações nos teores extraíveis de arsênio podem levar a uma maior bioacumulação em *E. andrei*.

Em um estudo conduzido por Vašíčková et al. (2016), as espécies *Enchytraeus crypticus* e *F. candida* foram utilizadas para avaliar a ecotoxicidade de um lodo de esgoto industrial contaminado por arsênio. Foram aplicadas diferentes quantidades de lodo (0,5%, 7,5% e 50%) em dois solos: solo A (pH = 6,4; carbono orgânico total = 1,2 %; CTC = 17,7 $cmol_c\ dm^{-3}$; argila 3,3 %) e solo B (pH = 7,2; carbono orgânico total = 4,2 %; CTC = 52,5 $cmol_c\ dm^{-3}$; argila 5,6 %). Os autores observaram que os organismos testes mostraram-se sensíveis ao aumento das concentrações de arsênio causadas pela adição do lodo ao solo. A taxa de aplicação de 0,5% não ocasionou efeitos sobre os organismos no solo A (As total = 7,7 mg kg^{-1}). Contudo, no solo B (teor total de As = 10 mg kg^{-1}), ocorreu mortalidade de 35% dos adultos de *E. crypticus* e 90% de *F. candida*. Esses autores evidenciaram que a toxicidade do lodo contaminado por arsênio é variável e depende dos atributos do solo, os quais devem ser levados em consideração ao avaliar os riscos associados à sua aplicação, pois nesse trabalho, nem todos os resultados puderam ser explicados apenas com base nas concentrações de arsênio. Essas duas espécies também foram utilizadas por González et al. (2011) para avaliar a toxicidade de um solo contaminado antes e depois de sua remediação (através da aplicação de materiais amenizantes). O solo utilizado apresentou pH ácido (pH = 3,06), baixa CTC (1,12 $cmol_c\ dm^{-3}$), baixo carbono orgânico (0,78 %) e contaminação múltipla por cádmio (6,2 mg kg^{-1}), cobre (46,5 mg kg^{-1}), chumbo (3.541 mg kg^{-1}), zinco (3.137 mg kg^{-1}) e arsênio (178 mg kg^{-1}). Nessas condições, não foi observada reprodução de *E. crypticus* e, para a espécie *F. candida*, a reprodução foi 80% menor que no solo controle. Após a remediação, os autores verificaram que os materiais utilizados na remediação ocasionaram mudanças nos atributos do solo (ex. pH e CTC) e reduziram a disponibilidade dos contaminantes. Isso resultou em menor toxicidade (sobrevivência de ambas as espécies e para reprodução de *E. crypticus*) aos organismos do solo.

Em outro estudo, Crouau e Moïa (2006) expuseram individualmente juvenis de *F. candida* durante 35 dias a um solo artificial (70% areia, 20% argila e 10% turfa; pH = 5) contaminado com um gradiente de concentrações de arsênio (0; 0,003; 0,009; 0,026; 0,08; 0,24; 0,74; 2,22; 6,67; 19,9 e 60 mg kg^{-1}). A concentração que causou redução de 50% na reprodução de *F. candida* é 2,19 mg kg^{-1}. Esse valor é muito menor que o observado nos trabalhos anteriormente citados e à primeira vista nos fazem questionar se o atual valor de prevenção para arsênio (15 mg kg^{-1}) adotado pelo Brasil é protetivo aos organismos do solo. Contudo, conforme observado, a toxicidade do arsênio é variável entre os solos,

principalmente em virtude da disponibilidade. Assim, espera-se que, em solos naturais, a toxicidade do arsênio seja menor.

Não foram encontrados trabalhos sobre os efeitos diretos do arsênio na reprodução ou sobrevivência dos ácaros da espécie *Hypoaspis aculeifer.* Assim, ao nosso conhecimento, este trabalho de tese foi o primeiro a testar a toxicidade do arsênio sobre esses organismos. Contudo, essa espécie tem sido utilizada em diversas avaliações ecotoxicológicas (HUGUIER et al., 2015), a saber: deltametrina, clorpirifós, dimetoato, cobre, NaCl, fenantreno, ácido bórico (OWOJORI; WASZAK; ROEMBKE, 2014), solo contaminados (MADANI et al., 2015) e vinhaça (ALVES et al., 2015).

2.3.3 Mecanismos de desintoxicação e proteção

Depois de o arsênio ser absorvido pelos organismos do solo, o contaminante circula através dos fluidos corporais até ser ligado a proteínas de transporte que o conduzirão a diferentes compartimentos (VIJVER et al., 2004), podendo ser particionados dentro de três grupos, sendo eles: biologicamente disponível, biologicamente indisponível e fração estocada (*storage*) (PEIJNENBURG et al., 1999). Os mecanismos de acumulação, regulação interna e toxicidade dos contaminantes são dependentes das espécies (PEIJNENBURG et al., 1999; VIJVER et al., 2004; SANTORUFO; VAN GESTEL; MAISTO, 2012) e o ponto de transição entre a ausência de efeito e os efeitos adversos é dado pelo teor limite da substância no organismo a qual é nominada como resíduo corporal crítico (*critical body residue*) (VIJVER et al., 2004). Segundo Romero-Freire et al. (2015), a capacidade máxima de acumulação de As corporal de *Eisenia andrei* é 1.019±167 μg As g^{-1} (peso seco), ao passo que maiores concentrações corporais são letais.

As duas principais formas de sequestro celular são através de inclusões corporais distintas (*distinct inclusion bodies*) e de ligações em proteínas termo estáveis (*heat-stable proteins*) (VIJVER et al., 2004). Essas estratégias evitam que haja a difusão do contaminante para outros tecidos (ARDESTANI; VAN STRAALEN; VAN GESTEL, 2014). As metalotieínas são proteínas (termo estáveis) ricas em grupos cisteína (contém enxofre) capazes de ligar-se com metais, que ocorrem em todo o reino animal e que estão associadas a desintoxicação e regulação de elementos-traço em organismos (CORTET et al., 1999; LANGDON et al., 2003; VIJVER et al., 2004).

Para o arsênio, há evidências que a complexação em proteínas ricas em enxofre é uma estratégia para reduzir o acúmulo de íons livres de As. Moriarty et al. (2009), ao investigarem a especiação de As em invertebrados coletados em sítios contaminados, verificaram que até 90% do As nos organismos estava reduzido e coordenado com enxofre. Lee et al. (2013), em *Eisenia fetida*, encontraram evidência de redução do As seguida pela complexação em grupos tiol. Essas evidências estão de acordo com a via metabólica do As em minhocas apresentadas por Langdon et al. (2003), a qual é seguida pelos passos: absorção de As^{+3} e As^{+5}, redução do As^{+5} para As^{+3}, complexação do As^{+3}-Tiol em proteínas ricas em enxofre, metilação para arsenobetaina, excreção organoarsênicas e eliminação através da urina, muco e outros.

No caso dos organismos de corpo duro, os contaminantes também podem ser depositados na cutícula e, quando ocorre a muda, eles são descartados com o antigo exoesqueleto (HUGUIER et al., 2015).

2.4 Valores de referência

Os valores orientadores da qualidade do solo são instrumentos de gestão ambiental utilizados na proteção da qualidade do solo, para disciplinar a entrada de contaminantes no solo, estimar efeitos adversos desses contaminantes sobre os seres vivos e determinar metas para a recuperação de áreas degradadas (CAETANO et al., 2016a, 2016b; NIVA et al., 2016). No Brasil, o Conselho Nacional do Meio Ambiente, através da Resolução CONAMA 420/2009, estabeleceu os valores orientadores da qualidade do solo (CONAMA, 2009). Foram estabelecidos três grupos de valores que podem ser classificados com base nos riscos de exposição aos contaminantes. No primeiro grupo estão os valores de referência de qualidade (VRQ). Eles representam os teores presentes em um solo considerado limpo, ou seja, são os teores naturalmente presentes ou de *background* geoquímico. O segundo grupo é composto pelos valores de prevenção (VP), os quais estabelecem os limites acima do qual a funcionalidade do solo pode ser comprometida. E, por fim, os valores de investigação (VI-agrícola, VI-residencial e VI-industrial) são os teores acima do qual há risco potencial à saúde humana. Nessa situação, o solo pode ser considerado contaminado e necessitará passar por processos de reabilitação.

Para o arsênio, o valor de prevenção, investigação agrícola, investigação residencial, investigação industrial são 15, 35, 55, 150 mg kg^{-1}, respectivamente (CONAMA, 2009). Esses valores são teores totais, e para a sua utilização, a Resolução CONAM 420/2009 recomenda

que a abertura das amostras seja realizada através de digestão ácida (método USEPA 3050b ou 3051) e as quantificações por técnicas espectrométricas.

Em relação aos valores de referência de qualidade (VRQ), a resolução CONAMA 420/2009 determinou que esses devem ser estabelecidos pelos órgãos ambientais competentes dos estados brasileiros. Oficialmente, os VRQ para arsênio nos Estados de Minas Gerais e São Paulo são 8 (COPAM, 2011) e 3,5 mg kg^{-1} (CETESB, 2016), respectivamente. Para os demais estados brasileiros, diversos pesquisadores têm publicado trabalhos que contribuirão para o estabelecimento dos VRQ para o arsênio ou outros elementos, a saber: Estado da Paraíba (ALMEIDA JÚNIOR et al., 2016), Município de Toledo no Paraná (JUCHEN et al., 2014), Estado do Paraná (MINEROPAR, 2005), Estado da Bahia (Rio Itapicuru) (DOS SANTOS et al., 2017), Estado de Santa Catarina (DE SOUZA et al., 2016), Estado do Rio Grande do Norte (PRESTON et al., 2014), Estado do Espírito Santo (PAYE et al., 2010), Estado do Mato Grosso (PIERANGELI et al., 2015), Bacia do Rio Doce (Minas Gerais) (GUEVARA et al., 2018) e solos brasileiros (FADIGAS et al., 2006).

Os valores de prevenção, no nível nacional, foram derivados tendo como base estudos de fitotoxicidade ou em avaliação de risco ecológico e foram adaptados da decisão de diretoria Nº195-2005-E de 2005 (CETESB, 2005; CONAMA, 2009). Esse documento (diretoria Nº195-2005-E de 2005), que serviu de referência para os valores orientadores, também determinou, dentre outros tópicos, que o relatório de "Estabelecimento de Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo" (CASARINI, 2001) continuasse em vigor. Nesse relatório, considerou-se como sendo o "valor de prevenção", a menor concentração de um contaminante que causasse fitotoxicidade e a concentração máxima permitida desses contaminantes presentes nos lodos de esgoto destinados à aplicação em solos agrícolas. Para o levantamento das concentrações que causassem fitotoxicidade, utilizou-se bibliografia nacional, enquanto que, para o segundo quesito, consultou-se a literatura internacional (CASARINI, 2001).

No Estado de Minas Gerais, os valores orientadores foram estabelecidos pela Deliberação Normativa Conjunta COPAM/CERH nº 02 de 2010. Posteriormente, a lista de valores orientadores foi alterada pela Deliberação Normativa COPAM nº 166 de 2011. Contudo, para os metais/ametais, à exceção de cádmio e chumbo, os valores de prevenção permaneceram os mesmos estabelecidos pela resolução 420/2009 e, consequentemente, os mesmos contidos no relatório de "Estabelecimento de Valores Orientadores para Solos e Águas Subterrâneas no Estado de São Paulo" (CASARINI, 2001). Segundo a Resolução

CONAMA 420/2009, os valores de prevenção podem ser revistos, desde que tecnicamente justificados, tendo a mesma base metodológica e assegurando o nível de risco.

Os valores de prevenção brasileiros podem ser considerados como critérios de qualidade do solo (CQS) fundamentados na garantia da funcionalidade do solo. Critérios de qualidade do solo para arsênio também são adotados em outros países e esses foram estudados por Zhou, Teng e Liu (2017). Esses autores apresentaram os diferentes CQS (para diferentes cenários de exposição) adotados para o arsênio em diferentes países/regiões. O quadro 1 apresenta os valores que foram levantados por Zhou, Teng e Liu (2017) considerando-se o uso agrícola do solo.

Quadro 1 - Critérios de qualidade do solo adotados por diferentes países.

País	Nome do critério de qualidade do solo	Critério de qualidade do solo considerando-se o uso agrícola do solo (mg kg^{-1})
Áustria	Trigger-value	20
Nova Escócia (Canadá)	Environmental quality standards for soil	17
Nunavut (Canadá)	Soil quality guideline	12
Ontário (Canadá)	Full depth background site condition standard	11
Estados Unidos	Eco-SSLs	18
Dinamarca	Soil quality criterium	10 ou 20
Holanda	Target value	29
Coréia	Soil contaminated protection level	6
Finlândia	Threshold value	5
Bélgica	RV ou TV	12 ou 15

Fonte: Adaptado de Zhou, Teng e Liu (2017).

Na Austrália, existem os "*ecological investigation level"* (EIL). Esses valores são utilizados nos primeiros níveis de uma avaliação de risco em ambientes terrestres. Para o arsênio, o teor de 20 mg kg^{-1} é adotado (REIMANN; DE CARITAT, 2017; REIMANN et al., 2018), contudo, quando se considera um envelhecimento do contaminante no solo de no mínimo 2 anos, o valor adotado é 40 mg kg^{-1} (OFFICE OF PARLIAMENTARY COUNSEL, 2013).

Na Itália e Alemanha, os critérios de qualidade do solo são denominados como "*Limit value"* e *"Trigger level"*, respectivamente. Para o arsênio, o valor adotado na Itália e Alemanha são 20 e 50 mg kg^{-1}, considerando-se os cenários de exposição "uso residencial" (BADERNA et al., 2015; ZHOU; TENG; LIU, 2017).

Conforme observado, o VP brasileiro para arsênio (15 mg kg^{-1}) encontra-se próximo aos valores de QCS adotados por outros países. Contudo, deve-se ter em mente que as condições edafoclimáticas brasileiras diferem dos países anteriormente mencionados e, desta forma, isso não garante que os VP adotados sejam adequados.

2.5 Curva de distribuição de sensibilidade de espécies

Dentre as metodologias utilizadas para estimar critérios de qualidade ambiental e em avaliações de risco ambiental, cita-se a construção de curvas de distribuição de sensibilidade de espécies (SSD) (FRAMPTON et al., 2006; POSTHUMA; SUTER; TRAAS, 2001). Dentre os países em que essa abordagem vem sendo utilizada, cita-se: alguns países da União Europeia, Estados Unidos da América, Canadá, Austrália e Nova Zelândia (DEL SIGNORE et al., 2016).

As SSD são funções usadas na descrição do comportamento de diferentes espécies expostas a um fator estressante (MALTBY et al., 2005). Delas, é possível obter o valor de HC_x (concentração perigosa a x% das espécies), i.e., o teor estimado de um contaminante em que x% de espécies contidas na curva de distribuição podem ser afetadas (BAIRD; VAN DEN BRINK, 2007; FRAMPTON et al., 2006). O valor de HC_5 tem sido considerado o critério de proteção mais comum, sendo uma concentração protetiva para a maioria das espécies (FRAMPTON et al., 2006; POSTHUMA; SUTER; TRAAS, 2001).

Não existe um consenso em relação ao número mínimo de espécies que devem compor a SSD, contudo, deve-se ter em mente que, quando menor o número de espécies, maior serão as incertezas dos valores de HCx gerados (DEL SIGNORE et al., 2016). Del Signore et al. (2016), em sua revisão sobre o desenvolvimento e aplicação das SSD em avaliação de risco ecológico, encontraram trabalhos que utilizaram no mínimo 4 dados.

Essa abordagem foi utilizada na derivação de critérios de qualidade da água (DU et al., 2015; QIE et al., 2017; ZHENG et al., 2017; LEWIS; THURSBY, 2018) e solo (SUN; PAN; ZHOU, 2012; OFFICE OF PARLIAMENTARY COUNSEL, 2013; DING et al., 2014; ZHOU; TENG; LIU, 2017) para arsênio.

Utilizando o teor total de arsênio, Sun, Pan e Zhou (2012) construíram SSD utilizando EC_{10} e EC_{50} para índices de crescimento (média aritmética dos *endpoints* medidos para uma mesma espécie em um mesmo experimento e posteriormente a média aritmética para uma mesma espécie em diferentes experimentos) de 28 espécies de plantas. A partir das SSD,

esses autores derivaram HC_5 e obtiveram valores de 7,83 e 25,3 mg kg^{-1} para EC_{10} e EC_{50}, respectivamente. Tendo como referência os valores derivados por Sun, Pan e Zhou (2012) e comparando-os com o VP para arsênio adotado pelo Brasil (15 mg kg^{-1}), espera-se que, em solos com teores iguais ao VP, menos de 5% das espécies de plantas podem ter seus atributos de crescimento afetados em 50%.

À primeira vista, acredita-se que o VP brasileiro para As seja suficientemente protetivo. Contudo, para se ter maior segurança sobre a adequabilidade, é necessário a realização de ensaios ecotoxicológicos utilizando solos brasileiros e incorporando-os a SSD. Por isso, esse trabalho de tese foi conduzido visando sanar parte das incertezas entorno da adequabilidade do VP para arsênio.

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SEGUNDA PARTE

ARTIGO 1 Selecting appropriate plant endpoints to evaluate ecotoxicity of arsenic in soils

Artigo redigido conforme normas específicas do periódico científico *Science of the Total Environment* (ISSN: 0048-9697). Este artigo é uma versão preliminar e, portanto poderão surgir alterações para adequá-lo. Os gráficos e tabelas foram inseridos no decorrer do texto para facilitar a leitura.

1 Selecting appropriate plant endpoints to evaluate ecotoxicity of arsenic in soils

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Abstract

Arsenic (As) is a metalloid that can be found in soils (originated from natural or human sources) and which is toxic to plants, representing an environmental concern. In a single laboratory experiment, plant growth test may provide many endpoints that can be used to evaluate the toxicity of a contaminant. However, only few of these endpoints are highly sensitive and reliable estimated (with low variability), which makes them more appropriate. In addition, for practical reasons, it is not always possible to measure many endpoints in a single test. For this reason, it is important to define which endpoints are most relevant. The sensitivity and reliability of an endpoint is highly related to test species, contaminant behavior, and soil properties. The objective of this study was to select appropriate endpoints to test the ecotoxicity of As based on plant growth trials. In order to achieve this, maize (*Zea mays*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), and radish (*Raphanus sativus*) plants were exposed to eight levels of As contamination (0; 8; 14.5; 26; 46.5; 84; 150; 270 mg kg^{-1}), in three different soils (Red-yellow Latosol - Oxisol, Haplic Cambisol - Inceptisol, and an artificial tropical soil - ATS). The endpoints measured were: early germination count (EGC), plant height (PH), relative leaf area (RLA), stem diameter (SD), total germination (TG), germination speed index (GSI), dry mass (DM), number of completely expanded leaves (CEL), plant survival (PS), soil plant analysis development chlorophyll level (SPAD), and final germination count (FnC). After EC_{50} values estimation for each endpoint, within each species and test soil, the endpoints were ranked according to their sensitivity (from the least to the greatest EC_{50} value) and reliability (from the least to the greatest amplitude in the 95% confidence intervals of the EC_{50}s estimated). The most sensitive endpoints were: EGC > RLA > DM > GSI, while the most reliable endpoints were: EGC > DM = GSI > PH, suggesting that EGC, RLA, and DM are the most adequate endpoints to be used in plant ecotoxicology studies with As.

Keywords: higher plant growth test, tropical soils, trace elements, phytotoxicity, sensibility, reliability.

1. Introduction

Arsenic (As) is a metalloid widely distributed throughout the Earth (Kabata-Pendias, 2011; Kabata-Pendias and Mukherjee, 2007) that can be originated from natural or human sources. Arsenic is a human carcinogen (Gamboa-Loira et al., 2017) and it is important to manage soil arsenic concentrations because plants may absorb and accumulate arsenic in edible parts, which represents a route for arsenic exposure (Islam et al., 2016; Zhao et al., 2010). Arsenic is also known to be toxic to plants (Kaur et al., 2012) and its toxicity depends on the speciation (arsenate, arsenite, and organic forms), concentration, exposure time, and, as reported for plants, the development stage of the organism and the physiological state (Kaur et al., 2012).

Plants have different capacities to absorb/accumulate As (Gulz et al., 2005; Wang et al., 2015) and differently tolerate this element (Meharg and Hartley-Whitaker, 2002; Yoon et al., 2015). Soil properties are known to influence availability of As and, consequently, accumulation and physiological effects of As to plants and, therefore, should be taken into account when evaluating its toxicity to plants (Melo et al., 2012; Yoon et al., 2015).

Laboratory ecotoxicological tests using standard species as test organisms have been used to characterize the risk associated to specific substances. The test organisms used are representative from different groups with different contaminant exposure pathways. Experiments carried out on plants have an important role in providing evidences of contaminant toxicity.

In a single standard test with plants, different endpoints, usually associated to biological events, growth (of specific organs or general) and death, can be measured. These endpoints are highly related to biomass production and generally associated to persistent and/or irreversible effects (OECD, 2006a). Depending on the particular nutritional needs and tolerance mechanisms of plant species, some organs can be more sensitive than others, showing early symptoms to specific contaminants (Garg and Singla, 2011). Furthermore, for practical reasons, it is not always possible to measure many endpoints in a single test. Therefore, the definition of the most adequate endpoints to be used in laboratory plant tests to evaluate the toxicity of specific contaminants is needed. Thereby, the objective of this study was to select endpoints that are best suited for As toxicology studies based on the sensitivity and reliability of endpoints measured in higher plant growth tests.

For this purpose, eleven endpoints were evaluated from six plant species: *Zea mays*; *Oryza sativa*; *Sorghum bicolor*; *Phaseolus vulgaris*; *Helianthus annuus*; and *Raphanus sativus,* which were exposed to gradients of increasing As concentrations in two natural soils (Red-yellow Latosol – Oxisol and Haplic Cambisol – Inceptisol) and an artificial soil (standard artificial tropical soil - ATS).

2. Materials and methods

2.1 Soils

The following soils were used in laboratory experiments: a Red-yellow Latosol - Oxisol collected in Itumirim (Minas Gerais) (21°17’08’’ E, 44°47’43’’ N), Brazil; an Haplic Cambisol - Inceptisol collected in Lavras (Minas Gerais) (21°13’46’’ E, 44°59’10’’ N), Brazil, these soils were collected at 0–20-cm depth. In addition, a substrate (artificial tropical soil – ATS) produced by mixing dry kaolinite clay (20%), fine sand (70%), and coconut fiber (10%) was utilized. The study soils were chosen to create a gradient of arsenic availability.

The soil samples were air dried and sieved to 2 mm. Soil characterization was performed according to the procedures described by Embrapa (EMBRAPA, 1997). Arsenic content of soils was determined using the Mehlich-1 extract, followed by measurements by graphite furnace absorption spectroscopy (Perkin Elmer - AAnalyst™) (EMBRAPA, 1997). Maximum water holding capacity (WHC) was determined as defined by ISO 11274 (ISO, 1998a). The method described in Campos et al. (2007) was employed to determine maximum arsenic adsorption capacity in natural soils (Oxisol and Inceptisol). Attributes of the soils are shown on Table 1.

Table 1. Physical and chemical properties of two natural soils (Inceptisol and Oxisol) and an artificial tropical soil (ATS) used in the laboratory higher plant growth tests. CEC – cation exchange capacity at pH 7; OM – organic matter content; P-rem – remaining phosphorous; AAC – maximum arsenic adsorption capacity; WHC – water holding capacity; Fe_2O_3 – Iron oxides.

Soil	Texture			pH (H_2O)	CEC	OM	P-rem	As	AAC	WHC	Fe_2O_3
	Clay	Silt	Sand								
	-------- % --------				*$cmol_c\ dm^{-3}$*	*%*	*mg L^{-1}*	*mg kg^{-1}*	*mg kg^{-1}*	*%*	*%*
ATS	19	8	73	5.2	2.27	7.84	36.1	<0.01	n.d.	74.0 ± 0.02	1.80 ± 0.10
Oxisol	26	8	66	4.4	0.27	0.24	6.84	0.026	714.3	41.0 ± 0.01	2.40 ± 0.00
Inceptisol	33	48	19	4.6	2.01	1.87	4.31	<0.01	1,667	59.0 ± 0.01	17.4 ± 1.03

n.d. - not determined.

Three days before starting the experiment, a fertilizer was applied in the all tested soils as defined by Malavolta (1980) for a 30-day period.

2.2 Test Plants

Six plant species were used in the higher plant growth tests to take into consideration exposure pathways of representative commercial crops. Three were monocotyledonous: maize (*Zea mays*), rice (*Oryza sativa*), sorghum (*Sorghum bicolor*); and three were eudicotyledonous: beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), and radish (*Raphanus sativus*).

2.3 Experimental procedure

Laboratory tests with plants were based on procedures described in OECD Test 208 (OECD, 2006b).

Arsenic was applied at concentrations of 0, 8, 14.5, 26, 46.5, 84, 150, and 270 mg kg^{-1} in order to cover the soil guideline values for As adopted by Brazil (CONAMA, 2009). Concentrations greater than 270 mg kg^{-1} were not used because they constitute risks to human health (CONAMA, 2009). The doses were prepared a stock solution of sodium arsenate dibasic heptahydrate ($Na_2HAsO_4 \cdot 7H_2O$, Sigma-Aldrich, purity ≥ 98%) in water. Different portions of this solution were directly added to the soils with different amounts of water depending on the intended dosage and with enough volume to obtain relative humidity of

50% of WHC of each soil. In addition, tests were carried out as saline control using sodium chloride (NaCl, Sigma-Aldrich, purity ≥ 99%). In this case, five doses equivalent to the ionic strength of the arsenate solutions, corresponding to 0, 8, 26, 84, and 270 mg kg^{-1}, were used.

Each experimental unit consisted of a plastic pot (Ø120 mm x 78 mm height) containing the equivalent of 500 g of dry soil weight (DW). Ten seeds of each species were planted in each experimental unit except for *Oryza sativa*, where fifteen seeds were used. Five replicates were carried out for the natural soils and three for ATS per test treatment.

Tests were carried out in Brazil under greenhouse with natural light between January and March. Average temperature during the experiment was 25 ± 3°C, which was controlled by an automatic ventilation system. Over the experiment, test vessels were watered daily in order to keep soil humidity at 50-60% of the WHC of the tested soils. Fourteen days after starting the test, one fifth of the fertilizer used at the beginning of the experiment was applied to each test unit in a nutritive solution mixed with the irrigation water.

The tests began with the seed sowing and lasted 21 days, after ≥50% of the seeds from control units had germinated. The endpoints measured in each test for each test species are presented and described in Table 2.

Table 2. Endpoints measured over the higher plant growth tests with six plant species and using two natural soils and an artificial tropical soil.

Endpoint	Description
Germination speed index (GSI)	Index determined through the following formula: $GSI = (E_1/N_1)+(E_2/N_2)+...(E_n/N_n)$ where E_1, E_2, E_n refers to the number of new germinated plants in the first (E_1) and final (E_n) count and N_1, N_2, N_n refers to the number of days after seeds sown (Maguire, 1962).
Early germination count (EGC)	Number of germinated plants immediately after ≥50% of the seeds from control units had germinated.
Final germination count (FnC)	Number of germinated plants after the test period described in Brasil (2009) for each test species.
Total germination (TG)	Maximum percentage of seeds that germinated over the test.

Plant survival (PS)	Difference between the maximum number of germinated plants (TG) and the number of plants at the end of the test (FnC).
Completely expanded leaves (CEL)	Total number of completely expanded leaves of all plants of each unit (determined one day before the end of the test) divided by the total number of plants.
Soil plant analysis development (SPAD)	Average of 30 readings carried out in the last completely expanded leaves of all plants of each unit, using a SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan). These readings were performed one day before the end of the test.
Relatively leaf area (RLA)	Leaf area of all leaves of one plant of each test unit (a plant representative of the average development of all plants of the test unit) determined using a LI-3100 Area Meter leaf meter (LI-COR, Lincoln, Nebraska, USA). These measurements were performed one day before the end of the test.
Stem diameter (SD)	Average stem diameter (measured 0.5 cm above soil surface using a digital pachymeter) of the harvested plants of each test unit measured at the end of the test.
Plant height (PH)	Average plant height of harvested plants (measured from the base to the point of the highest leaf) of each test unit at the end of the test.
Dry mass (DM)	Total weight of harvested plants after dried at 60 °C (until a constant weight) divided by the total number of plants harvested per test unit.

2.4 Statistical analysis

Whenever possible, the As concentration for 50% of effect (EC_{50}) was estimated for each endpoint by means of exponential, Gompertz, Hormesis, linear, and logistic models and using nominal As concentrations. The EC_{50} value selected for each endpoint was the one that had the greatest adjustment (r^2), the least amplitude of the 95% confidence intervals, and the smallest standard error. Assumptions about the selected model were validated by analyzing the regression residuals. The software used for these analyses was STATISTICA, version 7 (Statsoft, Tulsa, Oklahoma, 2011).

To evaluate the adequacy of the endpoints, the parameters measured in all test species were characterized according to its sensitivity (the higher the sensitivity, the lower the EC_{50} value) and reliability (the most reliable, the lower the amplitude of 95% confidence intervals of the EC_{50} estimated). Endpoints were ranked in two sequences per test species in each soil: i) one sequence ordering the endpoints in descending order of sensitivity; and, ii) another sequence ordering the endpoints in descending order of reliability. For each soil, and also in general (for all soils), endpoints were ordered by the mean ranks of sensitivity or reliability sequences (from the lower to the higher mean rank) among the test species. The endpoints with the lowest mean ranks were considered the most sensitive or reliable.

3. Results

In laboratory tests with plants all validity criteria defined in OECD Test 208 protocol were fulfilled and the endpoints measured in test units of saline controls did not show any statistical difference compared with control (Table 1, data enclosed).

The EC_{50} values estimated from As soil concentration for all species and soils are presented in Table 3. Overall, for the same parameter, the EC_{50} values followed the order of intensity levels: ATS < Oxisol < Inceptisol. For ATS, the data did not allow the estimation of the EC_{50} values for the endpoints PH and SPAD of *Z. mays* and for SPAD of *S. bicolor*. As observed in tests with ATS, in natural soils, the least sensitive species was *Zea mays*, with EC_{50} values estimated for four endpoints in the Oxisol and all the endpoints in the Inceptisol greater than the highest As tested dose. *P. vulgaris* was the most sensitive species with the lowest EC_{50} value estimated for EGC in the Oxisol (7.59 mg kg^{-1}). Among the monocotyledonous, *O. sativa* was the most sensitive species, with the lowest EC_{50} estimated for PH (21.0 mg kg^{-1}).

Table 3. EC_{50} values and respective 95% confidence intervals based on As nominal concentrations and derived for eleven endpoints (see Table 2 for endpoint codes and details) measured in higher plant growth tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), and radishes (*Raphanus sativus*) cultivated in artificial tropical soil (ATS), Oxisol, and Inceptisol, artificially contaminated with arsenic. Values are expressed in mg As kg^{-1} dry soil.

Soil	Endpoint	*O. sativa*	*Z. mays*	*S. bicolor*	*P. vulgaris*	*H. annuus*	*R. sativus*
ATS	EGC	33.8 (27.3 - 40.2)	91.3 (65.6 - 150)	50.0 (44.6 - 55.4)	< 8	18.8 (16.7 - 21.0)	17.3 (15.4 - 19.3)
	PH	21.0 (16.3 - 25.8)	n.e.	58.5 (43.6 - 73.4)	< 8	27.7 (26.0 - 29.5)	65.2 (51.4 - 79.1)
	RLA	30.5 (25.9 - 35.1)	58.4 (37.1 - 79.7)	33.3 (23.5 - 43.2)	< 8	28.5 (13.4 - 43.6)	57.8 (28.9 - 86.7)
	SD	36.9 (23.2 - 50.6)	109 (79.9 - 139)	61.5 (36.7 - 86.3)	< 8	27.5 (15.5 - 39.6)	92.7 (78.7 - 106)
	TG	78.7 (40.4 - 117)	101 (66.6 - 137)	92.2 (65.0 - 119)	< 8	31.9 (27.9 - 35.8)	47.4 (40.0 - 54.8)
	GSI	61.9 (39.6 - 84.1)	94.1 (60.4 - 127)	69.5 (52.7 - 86.3)	< 8	29.4 (27.4 - 31.4)	35.1 (48.4 - 69.9)
	DM	24.3 (19.1 - 29.5)	55.3 (30.7 - 79.9)	40.5 (30.5 - 50.5)	< 8	29.5 (26.8 - 32.2)	45.5 (40.8 - 50.2)
	CEL	33.2 (26.5 - 40.0)	62.4 (33.5 - 91.4)	57.3 (50.7 - 64.0)	< 8	33.6 (24.5 - 42.7)	103 (83.9 - 123)
	PS	45.0 (28.4 - 61.7)	39.4 (33.1 - 45.7)	43.2 (42.2 - 44.2)	< 8	32.8 (26.6 - 39.0)	59.1 (48.4 - 69.9)
	SPAD	44.4 (32.8 - 56.1)	n.e.	n.e.	< 8	35.0 (24.7 - 45.3)	109 (76.9 - 142)
	FnC	43.7 (31.8 - 55.6)	106 (62.6 - 150)	92.2 (65.0 - 119)	< 8	31.9 (27.9 - 35.8)	48.4 (40.4 - 56.4)
Oxisol	EGC	83.5 (79.9 - 87.2)	129 (97.2 - 161)	40.4 (20.7 - 60.2)	7.59 (5.43 - 9.76)	36.9 (32.3 - 41.4)	23.3 (17.6 - 28.9)
	PH	81.6 (72.2 - 90.9)	> 270	96.8 (85.5 - 108)	26.7 (21.9 - 31.4)	84.9 (73.8 - 96.0)	126 (107 - 145)
	RLA	88.0 (76.4 - 99.6)	118 (101 - 134)	69.0 (48.3 - 89.6)	16.8 (14.4 - 19.3)	104 (82.7 - 127)	113 (94.6 - 133)
	SD	113 (93.5 - 113)	> 270	153 (135 - 171)	38.9 (32.1 - 45.7)	119 (105 - 134)	120 (103 - 136)
	TG	217 (203 - 231)	166 (150 - 181)	152 (133 - 171)	17.2 (14.5 - 19.9)	84.3 (77.1 - 91.5)	84.8 (79.5 - 90.1)
	GSI	165 (155 - 175)	146 (126 - 166)	141 (124 - 158)	13.3 (11.0 - 15.7)	59.6 (51.1 - 68.2)	62.5 (55.6 - 69.5)
	DM	86.8 (80.6 - 93.0)	161 (129 - 193)	71.7 (55.1 - 88.2)	27.5 (23.2 - 31.9)	100 (85.5 - 115)	118 (102 - 134)
	CEL	144 (111 - 176)	> 270	179 (167 - 191)	38.1 (34.0 - 42.2)	119 (105 - 134)	109 (87.4 - 130)

	PS	137 (101 - 172)	144 (118 - 169)	195 (167 - 223)	41.1 (32.7 - 49.6)	121 (105 - 136)	124 (109 - 140)
	SPAD	146 (116 - 177)	> 270	169 (141 - 196)	40.8 (35.1 - 46.6)	140 (121 - 159)	128 (110 - 147)
	FnC	122 (98.4 - 146)	164 (150 - 178)	150 (131 - 170)	15.5 (12.3 - 18.6)	67.9 (59.6 - 76.3)	83.9 (77.5 - 90.3)
Inceptisol	EGC	193 (166 - 219)	> 270	212 (176 - 249)	27.1 (15.5 - 38.7)	221 (195 - 248)	123 (96.5 - 149)
	PH	200 (183 - 217)	> 270	251 (234 - 267)	74.3 (65.3 - 83.3)	> 270	> 270
	RLA	192 (155 - 228)	> 270	213 (186 - 241)	67.4 (56.5 - 78.2)	> 270	> 270
	SD	> 270	> 270	> 270	114 (69.9 - 158)	> 270	> 270
	TG	> 270	> 270	> 270	62.0 (56.1 - 67.9)	> 270	261 (239 - 282)
	GSI	> 270	> 270	242 (216 - 268)	54.2 (46.9 - 61.6)	> 270	211 (192 - 231)
	DM	229 (202 - 255)	> 270	218 (193 - 243)	64.9 (54.1 - 75.8)	> 270	> 270
	CEL	> 270	> 270	> 270	119 (105 - 133)	> 270	> 270
	PS	> 270	> 270	> 270	110 (74.9 - 146)	> 270	> 270
	SPAD	> 270	> 270	> 270	122 (105 - 139)	> 270	> 270
	FnC	> 270	> 270	256 (232 - 281)	53.0 (46.2 - 59.8)	> 270	248 (227 - 269)

n.e.= Data did not allow EC_{50} estimation.

In natural soils, the endpoints related to germination (EGC, FnC, TG, and GSI) showed a tendency to be more sensitive in eudicotyledonous species, and the endpoints related to growth (PH, RLA, SD, and DM) showed a tendency to be more sensitive in monocotyledonous species. For ATS, the observed tendencies were similar with the exception of *H. annuus*, which had lower EC_{50} values for endpoints related to growth (Table 3).

The most sensitive endpoints in tests with natural soils were EGC, RLA, and GSI; for ATS were DM, EGC, and RLA. Considering all soils, the most sensitive endpoints were EGC, RLA, and DM, while GSI, PH, and FnC had a median sensitivity. SPAD was always the least sensitive endpoint (Table 4).

Table 4. Germination and growth endpoints (see Table 2 for endpoint codes and details) sorted in descending order of sensitivity and respective average ranks (Rk) for all test species (from the endpoint with the lowest EC_{50} value to the endpoint with the highest EC_{50} value) to arsenic contaminated soils (artificial tropical soil – ATS, Oxisol, Inceptisol or all soils) in higher plant growth tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), and radishes (*Raphanus sativus*).

ATS		Oxisol		Inceptisol		All soils	
Endpoint	Rk	Endpoint	Rk	Endpoint	Rk	Endpoint	Rk
DM	3.5	EGC	1.3	EGC	2.0	EGC	2.3
EGC	3.7	RLA	4.0	RLA	4.9	RLA	4.3
RLA	3.8	GSI	4.2	GSI	4.9	DM	4.7
PH	4.8	FnC	4.5	FnC	5.3	GSI	5.1
PS	5.8	DM	5.2	DM	5.4	PH	5.5
GSI	6.2	PH	5.9	PH	5.9	FnC	5.6
SD	6.5	TG	6.3	TG	6.3	TG	6.7
CEL	6.5	SD	8.0	PS	7.6	PS	7.3
FnC	7.2	CEL	8.2	SD	7.8	SD	7.4
TG	7.5	PS	8.5	CEL	7.9	CEL	7.5
SPAD	9.0	SPAD	9.9	SPAD	8.1	SPAD	9.0

Concerning reliability, EC_{50} values estimated for eudicotyledonous species had generally the smallest 95% confidence intervals, being the most reliable ones.

The EC_{50} values for EGC often contained the shorter confidence intervals, followed by DM = GSI, PH, and FnC. The EC50 values estimated by SPAD, SD, and PS had generally the lowest reliability in all tested soils (Table 5).

Table 5. Germination and growth endpoints (see Table 2 for endpoint codes and details) sorted in descending order of reliability and respective average ranks (Rk) for all test species (from the endpoint with the EC_{50} value with the shortest amplitude of 95% confidence interval to the endpoint with the EC_{50} value with the largest 95% confidence interval) to arsenic contaminated soils (artificial tropical soil – ATS, Oxisol, Inceptisol or all soils) in higher plant growth tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), and radishes (*Raphanus sativus*).

ATS		Oxisol		Inceptisol		All soils	
Endpoint	Rk	Endpoint	Rk	Endpoint	Rk	Endpoint	Rk
DM	3.8	EGC	3.2	EGC	4.4	EGC	3.9
EGC	4.0	TG	3.7	PH	4.4	DM	4.9
PH	4.6	GSI	3.8	FnC	4.4	GSI	4.9
PS	4.9	FnC	4.5	GSI	4.8	PH	5.0
RLA	5.7	DM	5.5	DM	5.3	FnC	5.3
CEL	5.8	PH	5.9	TG	5.6	TG	5.4
GSI	6.1	RLA	7.0	RLA	5.8	RLA	6.2
FnC	6.8	SD	7.0	CEL	7.6	CEL	7.0
TG	7.0	CEL	7.5	SPAD	7.8	PS	7.2
SD	7.3	PS	8.7	PS	7.9	SD	7.5
SPAD	8.0	SPAD	9.3	SD	8.1	SPAD	8.3

4. Discussion

Since saline control tests did not show any toxic effect on the tested plants in any of the measured endpoints, the discussion of data gathered for higher plant growth tests will be safely based uniquely on As soil concentrations.

The different toxicity observed to As among the tested soil was due to contrasting attributes of the soils. The highest toxicity was observed for ATS because it contains lower iron oxides and clay content, and consequently greater availability of As when compared to natural soils (Campos et al., 2007; Zhao et al., 2009; Garg and Singla, 2011; Melo et al., 2012; Wang et al., 2015; Dai et al., 2016). Considering the level of organic material, the data

leads to the belief that the mineral makeup had a greater role in regulating the levels of phytoavailable arsenic than OM content in these soils (Table 1).

Germination is the first attribute that might be influenced by contaminants present in the soil and thus, the first plant attribute to be measured (Li et al., 2007). In the present study, early germination (EGC endpoint) was often the most sensitive endpoint responding to the presence of toxic elements in the soil, probably because some of the defense mechanisms of the plant are still not developed (Liu et al., 2005; Shri et al., 2009) and seeds are in direct contact with the soil solution that contains the contaminant (Wierzbicka and Obidzińska, 1998). In seeds, As might affect the balance between consumption and synthesis of NAD molecules and the reduction of the ATP/ADP ratio, which inhibits germination (Srivastava et al., 2013). In addition, the different levels of sensitivity to As observed between the test species in the endpoints related to germination could be due to intrinsic characteristics of each seed. For example, permeability of seed integument varies between species and is directly related to seed germination capacity when exposed to contaminants (Wierzbicka and Obidzińska, 1998).

In the present study, endpoints related to germination (EGC, FnC, and TG) had contrasting sensitivity and reliability. EC_{50} values estimated from EGC were the lowest ones (the most sensitive) and highly reliable, while those obtained from TG were the highest ones (the least sensitive) and moderately reliable (Tables 3, 4 and 5). Apparently, there used concentrations were not sufficiently high to inhibit germination but sufficiently high to retard germination.

Studies developed with *O. sativa* L. (Shri et al., 2009) and *Triticum aestivum* L. (Li et al., 2007; Liu et al., 2005) exposed to arsenic showed that growth parameters are more sensitive than germination parameters. Other authors even suggest that seeds are less sensitive to external toxicity because germination is more dependent on the energy reserves contained in cotyledons (Gong et al., 2001). Those suggestions agree with the results the present study, when comparing total germination data (TG) to growth data, but only for DM, RLA, and PH. On the other hand, the present study found that the EGC is more sensitive than any of the growth parameters (except in ATS; Table 4) and that even reliability is higher when considering the Oxisol and the three tested soils (Table 5). This indicates that initial stages of plant development are more prone to be affected by soil contamination with a low degree of variability associated.

After germination, subsequent metabolic processes become affected by soil contamination, which tends to be reflected in the development of plant organs and on growth endpoints (Srivastava et al., 2013). After being absorbed by roots, arsenate is reduced to arsenite, which is one of the main As speciation in plant tissue (Finnegan and Chen, 2012; Zhao et al., 2009). Consequently, during this chain of processes, reactive oxygen species are generated, leading to oxidative stress (Finnegan and Chen, 2012; Garg and Singla, 2011; Meharg and Hartley-Whitaker, 2002; Shri et al., 2009; Souza et al., 2014). In cells, As alters the flow of energy when ATP is converted into an unstable form of ADP-As (Finnegan and Chen, 2012; Garg and Singla, 2011; Kaur et al., 2012; Meharg and Hartley-Whitaker, 2002; Rosas-Castor et al., 2014) and affects carbon fixation reactions (Finnegan and Chen, 2012), as well as the DNA structure (Rosas-Castor et al., 2014). These facts might be the origin of the negative effect of As observed on the development of plant species, which was especially evident on relative leaf area and dry mass production (RLA and DM endpoints, respectively, which were the most sensitive measured growth endpoints).

The toxic effect of As on plant growth might also be explained by the lower nutrients acquirement (Finnegan and Chen, 2012; Kaur et al., 2012). The poor development of root structure caused by As (Li et al., 2007) interferes with the transport of ions in plant tissues. Arsenite (As^{3+}) can inhibit cell functions because it is linked to sulfhydryl groups of enzymes and proteins (Finnegan and Chen, 2012; Garg and Singla, 2011; Meharg and Hartley-Whitaker, 2002; Zhao et al., 2009). In addition, As^{3+} can inhibit cell division and stretching (de Freitas-Silva et al., 2016; Shaibur and Kawai, 2009). The combination of such effects affect plant development resulting in structural, metabolic, and visual modifications (de Freitas-Silva et al., 2016). On the other hand, the absorption of As^{5+} by roots takes place by means of phosphate transporters (Drličková et al., 2013a; Finnegan and Chen, 2012; Garg and Singla, 2011; Kumar et al., 2015; Rosas-Castor et al., 2014; Souza et al., 2014; Zhao et al., 2009). Thus, increasing concentrations of As leads to increasing competition for the same absorption sites of phosphate (Finnegan and Chen, 2012; Garg and Singla, 2011; Meharg and Hartley-Whitaker, 2002; Rosas-Castor et al., 2014), which can lead to phosphate deficiency in plants (depending on its availability in the growth medium). Phosphate deficiency causes a reduction in the number of leaves and in leaf expansion (Hawkesford et al., 2011). In addition to these processes, there is also an effect on carbon fixation (Finnegan and Chen, 2012), which explains the reduction in relative leaf area (RLA) observed in the test species of the present study. This endpoint was the second most sensitive and also contributed to the great

sensitivity of the DM endpoint (the third most sensitive endpoint), since the reduction of leaf area contributes to the decrease of the total mass production in plant.

Another effect of As in plants is related to chlorophyll degradation. Chlorophyll molecules can be degraded by plant cells as a source of carbon when insufficient carbohydrates are available (Finnegan and Chen, 2012). Apparently, in the present study, this phenomenon did not intensively occur or is not considerably sensitive to the presence of As in the test species used since the EC_{50} values estimated through chlorophyll measurements in leaves (SPAD) were generally higher when compared with other estimated toxic values (Table 3). This endpoint was the least sensitive and reliable among the tested soils (Tables 4 and 5). The low sensitivity of SPAD agrees with the data in the study conducted by Melo et al. (2012), who did not find visible changes on leaf coloration, which is highly related to chlorophyll content in *H. annuus* and *Ricinus communis* growing in exposed to an Entisol (4.6% of OM, pH of 4.4 and 72% of clay content) with 150 mg of As dm^{-3} of soil over 35 days that had reductions of 78% and 45%, respectively, in the shoot dry mass compared with the control. The low sensitivity of SPAD is also supported by Srivastava (2014), who exposed *Z. mays* seedlings to sodium arsenate solutions of 0, 100, and 200 µM (corresponding to 0, 7.5 and 15 mg As L^{-1}, respectively) and found that the measurements of chlorophyll *a* and *b* contents were considerably less sensitive than shoot length to As contamination. Since the studies of Melo et al. (2012) and Srivastava (2014) did not estimate toxicological values (EC_x) to As contamination, it is not possible to confirm the low reliability provided by SPAD in the present study.

5. Conclusion

Considering the results obtained in the laboratory tests and independently of the tested soil, the most sensitive endpoints, which is represented by endpoints that provided the lowest EC_{50} values, were early germination count > relatively leaf area > dry mass > germination speed index. The variables that had the highest reliability, providing EC_{50} values with the shortest amplitude of 95% confidence intervals, were early germination count > dry mass = germination speed index > plant height. The least sensitive and reliable endpoint was the soil plant analysis development (SPAD). Therefore, the use of data from the early germination count, relatively leaf area, and dry mass are the most adequate endpoints to be used in plant ecotoxicology studies with As. Although the dry mass endpoint had showed slightly lower

sensitivity than relatively leaf area, the use of dry mass may be preferred given its higher reliability and measurement easiness.

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ARTIGO 2 Effects of arsenic in the emergence and growth of plant species relevant to agroecosystems

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Effects of arsenic in the emergence and growth of plant species relevant to agroecosystems

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Abstract

Arsenic (As) is a metalloid toxic to humans, animals, and plants. One of the routes of As entrance into the food chain is through the consumption of edible parts of crops contaminated by As. Different plant species present distinctive As accumulation and tolerance capacities. These differences are also influenced by As availability in soils. Different soils might present higher/lower phytotoxic potential because of a high/low content of phytoavailable As. The aim of this work was to evaluate the effect of As contamination on plant emergence and initial growth, as well as the accumulated As content in different agricultural crops and to verify the protection level of the As prevention value adopted by Minas Gerais State, Brazil. Plants of *Zea mays*, *Oryza sativa*, *Sorghum bicolor*, *Phaseolus vulgaris*, *Helianthus annuus*, and *Raphanus sativus* were cultivated in three different soils (Red-Yellow Latosol - Oxisol, Haplic Cambisol - Inceptisol, and Artificial Tropical Soil - ATS) contaminated with increasing concentrations of As (0; 8; 14.5; 26; 46.5; 84; 150; 270 mg kg^{-1}). Early germination count, dry mass, As content, and bioconcentration were evaluated. The derived EC_{20} and EC_{50} values based on total As content were more variable among different soils than the corresponding EC_{20} and EC_{50} values based on extractable As content (i.e., phytoavailable), essentially due to different As availability among the tested soils. From the studied species, *P. vulgaris* was the most sensitive species and *Z. mays* was the least sensitive to As. Those species were the ones that accumulated the lowest As levels in shoot tissues. Arsenic concentrations measured in plant tissues and estimated bioaccumulation factors were not related to relative As toxicity among species. The high As levels measured in the tissues of some species (*H. annuus*, *O. sativa*, and *R. sativus*) emphasize the need to monitor this element in soils, mainly from agricultural areas, in order to prevent its entry into the food chain. In the present study, the current Brazilian prevention value for arsenic is adequate for soils with high arsenic adsorption capacity.

Keywords: arsenate, phytotoxicity, tropical soils, agronomic crops, bioaccumulation.

1. Introduction

From natural or anthropogenic origin, arsenic (As) exist in the environment in the form of inorganic and organic species (Cullen and Reimer, 1989; Finnegan and Chen, 2012; Kabata-Pendias, 2011; Kabata-Pendias and Mukherjee, 2007; Smith et al., 1998), usually having a tendency to accumulate in the soil. This element is not a nutrient and has been recognized as a toxic substance to many organisms, such as plants (Finnegan and Chen, 2012). It is known that depending on the concentration, it can cause decreased germination, inhibition of shoot growth, reduction of root growth, chlorosis, necrosis, leaf wilting, reduction of leaf area, reduction in photosynthetic rate, decrease in crop productivity and death of plants (de Freitas-Silva et al., 2016; Gulz et al., 2005; Liu et al., 2005; Shri et al., 2009; Smith et al., 2008; Souza et al., 2014).

Different plant species might not present the same effect when exposed to the same concentration of As (de Freitas-Silva et al., 2016; Farooq et al., 2016; Smith et al., 1998; Yoon et al., 2015) and As absorption and accumulation by plants are quite variable (Bhattacharya et al., 2010; Gulz et al., 2005; Wang et al., 2015). Plant species have different levels of metabolic tolerance and detoxification mechanisms of As compounds (Meharg and Hartley-Whitaker, 2002; Yoon et al., 2015). Plant species sensitivity to As is also related to the content of this element in the soil, its speciation, the exposure time to the contaminant, physiological status, and stage of plant development (de Freitas-Silva et al., 2016; Kader et al., 2016; Kaur et al., 2012).

It is known that for the same total As content, different soils can present different availability (Romero-Freire et al., 2014). Arsenic availability/mobility in the soil is closely related to soil chemical, physical, and mineralogical characteristics (Farooq et al., 2016; Meharg and Hartley-Whitaker, 2002; Melo et al., 2012; Yoon et al., 2015). Arsenic availability is highly dependent on soil mineralogy, partly due to the high affinity of As for iron compounds (Garg and Singla, 2011; Kabata-Pendias, 2011; Wang et al., 2015; Zhao et al., 2009).

For this reason, As phytotoxicity and the legislative threshold values allowed in the soil should not be established only based on the chemical determination of the total As content in the soil. Moreover, the guidelines values of soil quality currently adopted in Brazil are mostly based on the total As content in soil, not taking into account specificities inherent to organisms and local soils (CONAMA, 2009).

A research work was recently carried out aiming to evaluate the endpoints best suited to the study of As toxicity in crop plants, using two natural soils, an artificial tropical soil and six plant species (Martins et al. 2018, submitted). Following this study, the present research aims to evaluate As toxicity to different crop plants and in different soils, using the toxicity values estimated by the previously selected endpoints (the most sensitive and/or reliables to As: early germination count e dry mass) and to verify the adequacy of the protection level for the adopted As prevention value in Minas Gerais State, Brazil. We aim to contribute for the improvement the current database concerning risk assessment of As (ecological and human health) in tropical soils.

2. Material and methods

2.1 Soils and plant tests

The following soils were used for the laboratory test: i) Red-Yellow Latosol (Oxisol), collected in Itumirim, Minas Gerais, Brazil (Latitude E-21°17’08’’, Longitude N-44°47’43’’); ii) Haplic Cambisol (Inceptisol) collected in Lavras, Minas Gerais, Brazil (Latitude E-21°13’46’’, Longitude N-44°59’10’’); and iii) Artificial Tropical Soil (ATS) produced by mixing kaolinite clay (20%), fine sand (70%) and coconut fiber (10%). The natural soils were collected at 0–20-cm depth. The soil attributes are presented in Table 1 and were determined according to standard procedures (EMBRAPA, 1997). The maximum water retention capacity was established according to ISO 11274 (1998). The maximum adsorption capacity of As for the natural soils (Oxisol and Inceptisol) was determined according to the methodology described by Campos et al. (2007).

Table 1. Physical and chemical attributes of the Artificial Tropical Soil (ATS) and two natural soils (Inceptisol and Oxisol) used in the laboratory ecotoxicological tests (data was obtained from Martins et al. 2018, submitted). CEC – Cation exchange capacity in pH 7; OM - Organic matter; P-rem – Remaining phosphorus; AAC – Maximum arsenic adsorption capacity; WHC - Maximum water retention capacity; Fe_2O_3 – Iron oxides. n.d. = not determined.

Soils	ATS	Oxisol	Inceptisol
pH (H_2O)	5.2	4.4	4.6
CEC ($cmol_c\ dm^{-3}$)	2.27	0.27	2.01
OM (%)	7.84	0.24	1.87
P-rem ($mg\ L^{-1}$)	36.1	6.84	4.31
AAC ($mg\ kg^{-1}$)	n.d.	714.3	1,667
WHC (%)	74.0 ± 0.02	41.0 ± 0.01	59.0 ± 0.01
Texture			
Sand (%)	73	66	19
Silt (%)	8	8	48
Clay (%)	19	26	33
Fe_2O_3 (%)	1.80 ± 0.10	2.40 ± 0.00	17.4 ± 1.03

Six plant species representative of tropical agroecosystems - and with a focus on staple crops - were used in the laboratory trials. Such plants comprised three species of monocotyledonous plants - maize (*Zea mays*), rice (*Oryza sativa*), and sorghum (*Sorghum bicolor*) - and three eudicotyledons plants - beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*) and radish (*Raphanus sativus*).

2.2 Experimental procedure

Laboratory tests with plants were based on procedures described in OECD Test 208 (OECD, 2006b). Soil samples were previously air dried and sieved (<2 mm). Three days before the beginning of the experiment, the soil was fertilized with nutrient rates proportional to the experimental period, by applying N = 100 mg; P = 100 mg; K = 50 mg; Ca = 37 mg; Mg = 15 mg; S = 25 mg; B = 0.25 mg; Cu = 0.75 mg; Zn = 2.5 mg; Mn = 5 mg; and Mo = 0.1 mg per kg^{-1} dry soil (Malavolta, 1980).

In each test soil, the concentration gradients of 0, 8, 14.5, 26, 46.5, 84, 150 and 270 mg As kg^{-1} were used in order to cover soil quality guidelines values adopted Brazil (CONAMA, 2009). Doses higher than 270 mg kg^{-1} were not used since they already present potential risks to humans (CONAMA, 2009). Arsenic doses were applied by preparing a stock solution of sodium arsenate dibasic heptahydrate (Na_2HAsO_4 $7H_2O$, purity ≥ 98%, Sigma-Aldrich, St. Louis, MO, USA) in water. The As solution was directly added to the soil in different proportions, along with different amounts of water in order to obtain 50% of water holding capacity (WHC) of each experimental soil. Samples from each dose of the three tested soils were collected for chemical analysis of total and extractable (phytoavailable) levels of As in the soil.

Each experimental unit of the laboratory tests consisted of a plastic pot (Ø 120 mm x 78 mm height) containing the equivalent of 500 g dry soil. In each experimental unit, ten seeds of each species were sown, except for *Oryza sativa*, in which 15 seeds were used. Five replicates were performed for the natural soils (Oxisol and Inceptisol) and three replicates for ATS.

The experiments were conducted under the greenhouse with natural light between the months January and March. The average temperature during the experiment was 25 ± 3°C, which was controlled by an electronic ventilation system. Over the experiment, soil moisture was kept between 50-60% of the WHC by daily watering aiming at compensating evapotranspiration. Plant weight was considered negligible compared to soil weight.

The test started with sowing and lasted 21 days after emergence of at least 50% of the plants from the control treatment (0 mg As kg^{-1}). After 14 days from the beginning of the test, one fifth of the initial fertilization was supplied as cover fertilizer, applied as a nutrient solution along with the irrigation water. The endpoints considered in each test for analysis of As toxicity were ‘Early Germination Count’ (EGC),which represents the number of plants germinated immediately after ≥ 50% of the seeds from control units had germinated; and ‘Dry mass’ (DM). At the end of the test, the plants of each experimental unit were cut at the base and dried at 60°C until constant weight for the subsequent determination of dry mass for plant shoot divided by the total number of plants per experimental unit. The biomass in each experimental unit was used to analyze As contents in plant tissues.

159 2.3 Chemical analysis of arsenic in soil and plants
160
161 Total and extractable As contents were measured for each collected soil.
162 Samples were oven dried at 40°C until constant weight, grinded in mortar and agate
163 pestle and passed through a 150 μm nylon sieve. For the total contents, samples were
164 digested using HNO_3 (65%) in a microwave oven (Mars 5, CEM Corporation,
165 Matthews, NC, USA) (USEPA, 2007). For the extractable (phytoavailable) contents,
166 suspensions of 0.5 g of each sample and 5 mL of Mehlich-1 solution (0.05 mol L^{-1} HCl
167 + 0.0125 mol L^{-1} H_2SO_4) were shaken for 5 min, left resting for 16 hours and filtered
168 through a 0.45-μm filter (EMBRAPA, 1997).Arsenic concentrations were determined
169 by atomic absorption spectrophotometry with electrothermal atomization using graphite
170 furnace (Perkin Elmer – AAnalyst™ 800, Norwalk, CT, USA).
171 Plant tissue samples were dried, weighed, milled, digested and analyzed using
172 the same procedures adopted for the determination of total As contents in soil samples.
173 The bioconcentration factor was determined from the ratio between the As
174 concentration in shoot and the total As concentration measured in the soil.
175 To verify the accuracy for determination of As content in plant tissue and soil, in
176 each battery of analysis, certified reference materials containing matrices compatible
177 with the analyzed samples were used. For plant material BCR-482 No. 638 (Lichen –
178 As: 0.85 ± 0.07 mg kg^{-1}) and for soil samples SRM 2711a (Montana II soil – As: 107 ±
179 5 mg kg^{-1}) and SRM 2710a (Montana I soil – As: 1540 ± 100 mg kg^{-1}) were used.
180 In addition, blank samples were used for control and determination of the
181 detection limits and method quantification limits (Penha et al., 2017).
182
183 2.4 Statistical Analysis
184
185 Whenever possible, for the variables EGC and DM, the concentration of As that
186 could cause the reduction by 20% and 50% (EC_{20} and EC_{50}, respectively) in relation to
187 the control treatment were derived. For this, non-linear regression models were applied
188 using the total and extractable As concentrations. The exponential, Gompertz, Hormesis
189 and logistic models were tested. The selected EC_{20} and EC_{50} for each variable were
190 chosen based on adjustments (R^2), the lowest amplitudes of the confidence limits and
191 the lowest standard errors of the mean. The assumptions of the selected models were
192 validated by normality analysis of regression residuals. For each soil, the EC_{50} and EC_{20}

values were used to build a species sensitivity distribution curve (SSD) to estimate hazard concentration values with 50 and 5% of protection level (HC_{50} and HC_5, respectively). HC_5 was compared to the current prevention value for As, which was considered appropriate when it was smaller than HC_5.

The differences in the As contents in shoot and in the bioconcentration factor between the different doses of each gradient were analyzed by one-way ANOVAs followed by Tukey post hoc test ($\alpha = 0.05$). When necessary, the data was transformed (Log_{10} (X+1) or square root (X+1)) to obtain normality and homogeneity of data variance.

The softwares STATISTIC, version 7 (Statsoft, Tulsa, OK, 2011), ETX 2.0 software (Vlaardingen et al., 2004) and SISVAR (Ferreira, 2014) were used for statistical analysis.

3. Results

3.1 As concentration in soil

The recovery rate of the analytical standards BCR-482, SRM 2711a and SRM 2710a were, on average, 76.5%, 98.2% and 101%, respectively. Total and extractable As contents are presented in Table 2. The average As recovery was 87.3 ± 12.5% and the lowest value was 51.5% observed for the treatment 14.5 mg kg^{-1} in the Inceptisol. For extractable levels of As, the recovery rate of the nominal value followed the decreasing order: ATS > Oxisol > Inceptisol, with a mean recovery of 31.2 ± 14.4%, 16.1 ± 6.91% and 2.22 ± 1.03% for ATS, the Oxisol and the Inceptisol, respectively. In general, for the three tested soils, the recovery rate of the extractable contents was higher with the increase of As doses.

Table 2. Applied and mean contents (mean ± standard deviation, *n* = 3) of total and extractable As (expressed in mg kg^{-1}) measured in Artificial Tropical Soil (ATS), Oxisol and Inceptisol used in the phytotoxicological tests and respective percentage means (mean ± standard deviation, *n* = 3) of the applied contents.

	Applied (mg kg^{-1})	Total (mg kg^{-1})	% of nominal	Extractable (mg kg^{-1})	% of nominal
ATS	0	3.10 ± 0.26	-	0.33 ± 0.09	-
	8	7.18 ± 1.61	89.8 ± 20.1	1.17 ± 0.44	14.6 ± 5.52
	14.5	14.3 ± 0.91	98.8 ± 6.29	2.82 ± 0.82	19.5 ± 5.67
	26	25.3 ± 1.23	97.4 ± 4.72	4.62 ± 1.83	17.8 ± 7.02
	46.5	40.6 ± 4.13	87.2 ± 8.87	18.8 ± 0.98	40.5 ± 2.12
	84	80.4 ± 19.4	95.7 ± 23.1	29.3 ± 9.41	34.9 ± 11.2
	150	148 ± 6.45	99.1 ± 4.30	56.9 ± 6.24	37.9 ± 4.16
	270	260 ± 88.3	96.6 ± 32.7	145 ± 13.6	53.8 ± 5.06
Oxisol	0	2.43 ± 0.30	-	0.37 ± 0.07	-
	8	5.57 ± 1.00	69.6 ± 12.5	0.64 ± 0.08	8.08 ± 1.06
	14.5	12.0 ± 0.30	83.2 ± 2.06	1.72 ± 0.16	11.8 ± 1.14
	26	21.8 ± 1.13	83.8 ± 4.36	3.34 ± 0.35	12.8 ± 1.35
	46.5	40.1 ± 8.59	86.1 ± 18.5	7.47 ± 4.57	16.0 ± 9.83
	84	69.2 ± 17.3	82.3 ± 20.6	14.8 ± 1.10	14.6 ± 1.32
	150	134 ± 9.24	89.3 ± 6.16	31.6 ± 3.20	21.1 ± 2.13
	270	247 ± 40.4	91.8 ± 15.0	78.4 ± 13.0	29.0 ± 4.82
Inceptisol	0	1.68 ± 1.09	-	0.21 ± 0.08	-
	8	7.87 ± 1.93	98.4 ± 24.2	0.10 ± 0.01	1.29 ± 0.15
	14.5	7.47 ± 0.85	51.5 ± 5.86	0.21 ± 0.08	1.44 ± 0.60
	26	22.7 ± 0.41	87.2 ± 1.58	0.48 ± 0.10	1.86 ± 0.39
	46.5	32.3 ± 12.0	69.4 ± 25.8	1.17 ± 0.16	2.52 ± 0.35
	84	87.4 ± 3.39	104 ± 4.03	1.42 ± 0.23	1.69 ± 0.28
	150	113 ± 7.43	75.8 ± 4.95	3.69 ± 0.35	2.46 ± 0.23
	270	257 ± 14.7	95.3 ± 5.46	11.6 ± 1.58	4.31 ± 0.59

3.2 Toxicity values

Overall, the EC_{20} and EC_{50} values derived from the total contents followed the order: ATS < Oxisol < Inceptisol (Table 3). Exceptions were observed for EC_{20} from *S. bicolor* and *R. sativus* and EC_{50} from *S. bicolor* derived from EGC, where the toxicity values were higher for ATS when compared to Oxisol (Table 4).

For the EC_{20} and EC_{50} values derived from the extractable contents no clear trend was observed. The toxicity values measured in the different tested soils were often closer, with a frequent overlap between the confidence intervals of the toxicity values within the same species among the tested soils (Table 4).

Table 3. EC_{20} (*effect concentration of* 20%) and EC_{50} (*effect concentration of* 50%) values and confidence limits of 95% for early germination count (EGC) and dry mass (DM), based on total arsenic concentrations in tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*), and radish (*Raphanus sativus*) in ATS, Oxisol and Inceptisol artificially contaminated by arsenic with increasing concentrations. Values are expressed as mg kg^{-1}.

Soil	Species	EGC		DM	
		EC_{20}	EC_{50}	EC_{20}	EC_{50}
ATS	*O. sativa*	15.7 ± 8.60	31.5 ± 5.95	15.3 ± 6.86	24.0 ± 4.45
	Z. mays	72.8 ± 20.4	104 ± 14.4	29.0 ± 23.9	49.9 ± 25.4
	S. bicolor	36.3 ± 13.0	44.0 ± 5.10	19.1 ± 11.9	38.9 ± 10.3
	P. vulgaris	<7.18	<7.18	<7.18	<7.18
	H. annuus	11.6 ± 2.90	18.5 ± 2.10	21.9 ± 3.80	27.1 ± 5.90
	R. sativus	9.96 ± 4.05	16.9 ± 2.65	25.1 ± 6.15	41.9 ± 5.25
Oxisol	*O. sativa*	52.2 ± 6.80	69.1 ± 4.20	50.8 ± 7.75	72.7 ± 8.05
	Z. mays	81.0 ± 27.3	111 ± 32.6	91.5 ± 27.7	143 ± 30.5
	S. bicolor	14.1 ± 8.35	31.3 ± 19.2	19.6 ± 12.8	60.8 ± 14.7
	P. vulgaris	N.E.	5.21 ± 4.24	13.4 ± 7.13	20.3 ± 4.25
	H. annuus	25.4 ± 3.55	31.8 ± 4.60	57.0 ± 15.0	84.3 ± 16.0
	R. sativus	8.17 ± 5.65	18.3 ± 7.85	78.4 ± 21.5	95.0 ± 42.0
Inceptisol	*O. sativa*	97.2 ± 30.1	175 ± 27.0	129 ± 36.5	189 ± 33.5
	Z. mays	>257	>257	179 ± 49.0	>257
	S. bicolor	124 ± 49.4	176 ± 43.5	122 ± 33.0	183 ± 30.0
	P. vulgaris	14.7 ± 9.70	20.5 ± 12.8	24.7 ± 11.2	46.7 ± 7.95
	H. annuus	117 ± 30.5	202 ± 33.0	>257	>257
	R. sativus	56.5 ± 25.8	110 ± 22.8	161 ± 144	>257

N.E.= Data obtained did not permit ECx to be estimated.

Table 4. EC_{20} (*concentration effect of 20%*) and EC_{50} (*concentration effect of 50%*) values and 95% confidence limits for early germination count (EGC) and dry mass (DM), based on arsenic extractable concentrations in tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*) and radish (*Raphanus sativus*) in ATS, Oxisol and Inceptisol artificially contaminated by arsenic with increasing concentrations. Values are expressed as mg kg^{-1}.

Soil	Species	EGC		DM	
		EC_{20}	EC_{50}	EC_{20}	EC_{50}
ATS	*O. sativa*	2.79 ± 2.21	7.84 ± 2.99	3.20 ± 1.38	4.45 ± 0.49
	Z. mays	26.9 ± 6.95	38.6 ± 5.70	16.5 ± 9.57	22.8 ± 0.51
	S. bicolor	14.7 ± 3.00	19.8 ± 1.60	N.E.	8.55 ± 0.51
	P. vulgaris	<1.17	<1.17	<1.17	<1.17
	H. annuus	2.48 ± 0.51	3.58 ± 0.41	2.71 ± 1.00	5.69 ± 0.50
	R. sativus	2.08 ± 0.72	3.29 ± 0.40	10.2 ± 4.87	19.2 ± 0.50
Oxisol	*O. sativa*	10.4 ± 1.63	14.7 ± 1.10	10.2 ± 1.91	15.6 ± 0.48
	Z. mays	17.7 ± 6.90	25.7 ± 9.80	19.7 ± 7.95	36.2 ± 0.50
	S. bicolor	2.67 ± 1.94	4.85 ± 4.65	2.89 ± 2.56	12.4 ± 0.49
	P. vulgaris	N.E.	0.44 ± 0.34	1.81 ± 1.26	3.13 ± 0.50
	H. annuus	4.03 ± 0.73	5.42 ± 1.03	12.5 ± 3.62	18.4 ± 0.50
	R. sativus	N.E	2.75 ± 1.47	17.1 ± 5.75	21.2 ± 0.50
Inceptisol	*O. sativa*	2.68 ± 1.53	5.86 ± 1.43	3.38 ± 2.05	7.80 ± 0.50
	Z. mays	>11.6	>11.6	8.18 ± 3.74	>11.6
	S. bicolor	3.87 ± 2.31	6.64 ± 2.36	4.44 ± 2.19	6.94 ± 0.50
	P. vulgaris	N.E.	0.53 ± 0.42	1.09 ± 0.13	1.34 ± 0.45
	H. annuus	3.13 ± 1.42	7.71 ± 1.87	>11.6	>11.6
	R. sativus	1.36 ± 1.35	2.92 ± 1.01	N.E.	>11.6

N.E.= Data obtained did not permit ECx to be estimated.

The species sensitivity distribution curves and the HC_5 and HC_{50} values are presented in Figure 1 and Table 5.

The species *P. vulgaris* was the most sensitive to As in all soils tested. It was not possible to calculate EC_{20} and EC_{50} values for ATS due to lack of germination even in the lowest dose. In this case, the lowest tested concentration was considered for construction the SSD. On the other hand, *Z. mays* was the least sensitive species to As in all tested soils.

For Inceptisol, in some cases, *Z. mays* and *H. annuus* presented EC_{20} and EC_{50} values higher than the highest tested dose. Therefore, the highest dose was used for SSD curve construction. In this soil no HC_x values were derived for EC_{50} because the model was not validating due to lack of data normality.

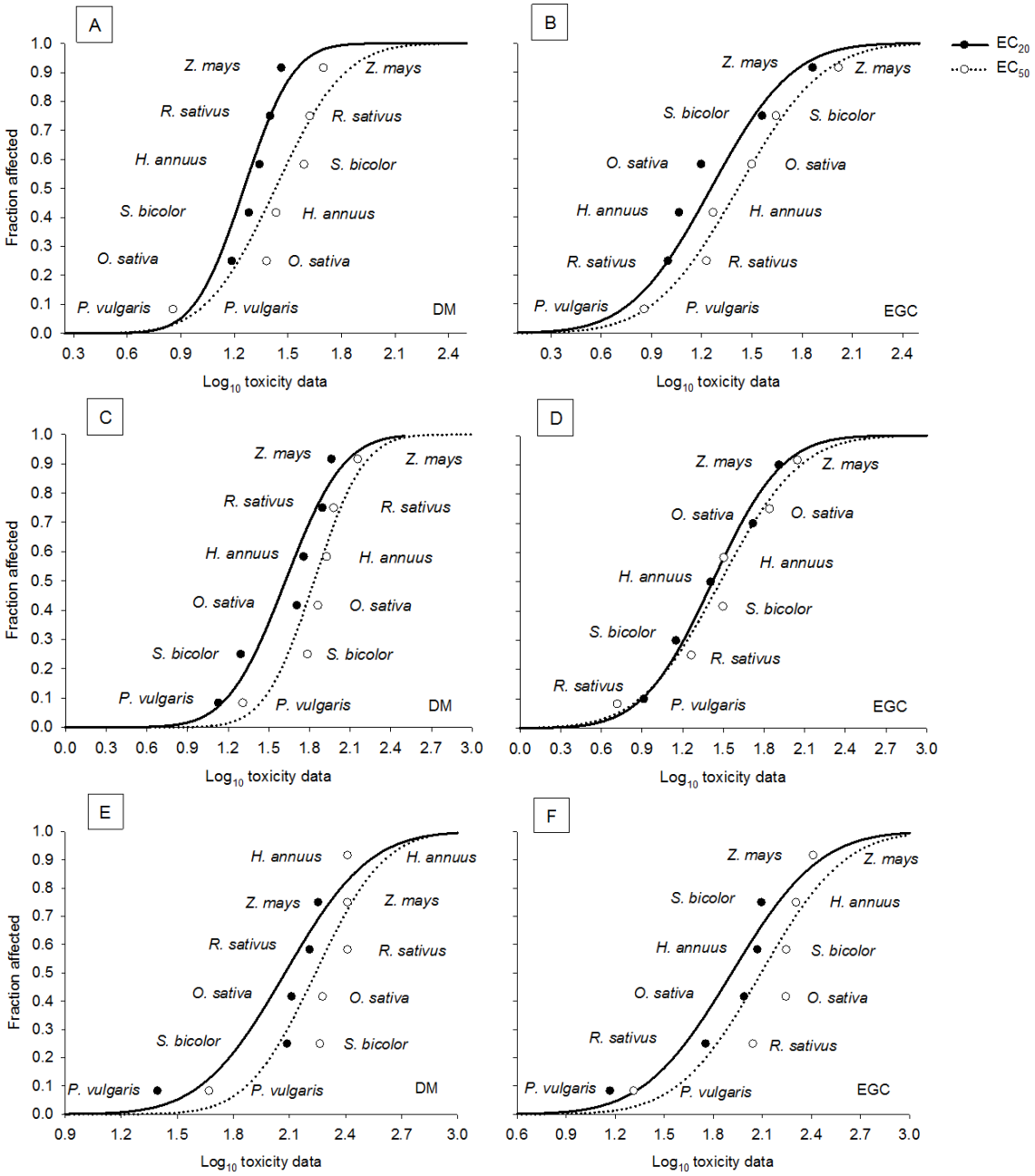

Figure 1. Species sensitivity distribution curves based on EC_{20} (first line of graphs) and EC_{50} values (second line of graphs) estimated through data of early germination count (EGC) and dry mass (DM) in tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*) and radish (*Raphanus sativus*) in ATS (A and B), Oxisol (C and D) and Inceptisol (E and F) artificially contaminated by arsenic with increasing concentrations. Note: The species at the bottom of the curves were considered more sensitive than those at the top.

Table 5. Comparison between current prevention value (PV_{As}) considered for arsenic in Minas Gerais State, Brazil (CONAMA, 2009) and the hazardous concentration (HC) values at 5 and 50% (HC_5 and HC_{50}, respectively) estimated from EC_{20} and EC_{50} values (based on total and available As concentration) considering data of early germination count (EGC) and dry mass (DM) in tests with rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), sunflower (*Helianthus annuus*) and radish (*Raphanus sativus*) in ATS, Oxisol and Inceptisol artificially contaminated with increasing arsenic concentrations. Values are expressed in mg of As kg^{-1} of dry soil.

		ATS			
		Dry mass		Early germination count	
		Total	Available	Total	Available
HC_5	EC_{20}	7.47 (2.80 - 11.5)	0.66 (0.05 - 1.85)	3.88 (0.69 - 8.36)	0.50 (0.04 - 1.47)
	EC_{50}	7.85 (1.98 - 14.5)	1.03 (0.12 - 2.67)	5.27 (0.87 - 11.7)	0.70 (0.05 - 2.15)
HC_{50}	EC_{20}	17.9 (11.8 - 27.0)	4.43 (1.60 - 12.2)	18.0 (8.76 - 37.1)	4.33 (1.57 - 11.9)
	EC_{50}	26.9 (15.0 - 47.9)	6.93 (2.83 - 16.9)	26.2 (12.3 - 55.6)	6.59 (2.29 - 18.9)
		Oxisol			
HC_5	EC_{20}	10.7 (2.36 - 21.2)	1.38 (0.19 - 3.28)	4.97 (0.51 - 12.2)	1.37 (0.07 - 3.50)
	EC_{50}	21.4 (5.88 - 38.3)	3.36 (0.66 - 6.93)	4.61 (0.56 - 11.7)	0.39 (0.02 - 1.38)
HC_{50}	EC_{20}	41.9 (22.1 - 79.4)	7.79 (3.45 - 17.5)	26.2 (10.7 - 63.8)	6.67 (2.41 - 18.4)
	EC_{50}	68.4 (39.7 - 117)	14.2 (7.24 - 28.2)	29.9 (12.4 - 72.3)	4.78 (1.48 - 15.4)
		Inceptisol			
HC_5	EC_{20}	28.5 (5.78 - 58.4)	0.85 (0.09 - 2.05)	14.9 (2.22 - 34.9)	0.87 (0.13 - 1.84)
	EC_{50}	-	1.60 (0.31 - 3.34)	-	0.60 (0.06 - 1.58)
HC_{50}	EC_{20}	119 (60.9 - 233)	4.34 (1.81 - 10.3)	81.8 (36.7 - 182)	3.48 (1.65 - 7.31)
	EC_{50}	-	6.95 (3.49 - 13.8)	-	4.18 (1.68 - 10.4)
PV_{As}		15			

3.3 Arsenic content in plants

It was not possible to determine the As content in dry mass, and consequently calculate bioconcentration factors in some treatments due to insufficient amount of biomass for analysis (Table 6). This was observed mainly for ATS due to greater phytotoxicity.

Overall, the levels of As within the same species were higher for ATS, followed by Oxisol and Inceptisol. An exception was observed for *R. sativus*, where the As contents of the plants cultivated in Oxisol were higher than ATS (Table 6). Similarly to As concentration in plants, the bioaccumulation factors (BFs) tended to be higher for ATS, followed by Oxisol and Inceptisol (Table 7).

The increase of arsenic soil concentrations resulted in rise of arsenic concentration in plant shoot, but an opposite behaviour was observed for the bioconcentration factor, which presented a reduction. The As contents and the bioconcentration factors did not allow the separation of the species of monocotyledons from the species of eudicotiledoneas.

Table 6. Mean arsenic concentration (mean ± standard deviation, n = 3 to 5) in shoots of rice (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), radish (*Raphanus sativus*) and sunflower (*Helianthus annuus*) cultivated in ATS, Oxisol and Inceptisol artificially contaminated with increasing arsenic concentrations. Values are expressed as mg kg^{-1}.

Dose	*O. sativa*			*Z. mays*			*S. bicolor*		
	ATS	Oxisol	Inceptisol	ATS	Oxisol	Inceptisol	ATS	Oxisol	Inceptisol
0	0.35 ± 0.29 c	0.31 ± 0.21 b	0.17 ± 0.06 d	0.08 ± 0.04 c	0.11 ± 0.03 d	0.07 ± 0.03 e	< 0.06 c	0.07 ± 0.02 e	0.07 ± 0.02 e
8	5.12 ± 0.87 b	1.71 ± 0.07 a	0.50 ± 0.04 cd	1.22 ± 0.05 ab	0.71 ± 0.12 c	0.38 ± 0.07 d	1.12 ± 0.11 bc	0.92 ± 0.07 d	0.24 ± 0.07 de
14.5	5.20 ± 1.41 b	2.25 ± 0.25 a	0.78 ± 0.06 c	1.22 ± 0.03 ab	0.67 ± 0.05 c	0.54 ± 0.17 cd	1.36 ± 0.42 b	1.18 ± 0.05 cd	0.42 ± 0.09 cd
26	24.9 ± 0.13 a	3.09 ± 0.58 a	0.89 ± 0.09 c	2.54 ± 2.72 b	0.65 ± 0.14 cd	0.87 ± 0.07 bc	1.95 ± 0.18 b	1.22 ± 0.19 bcd	0.81 ± 0.16 bc
46.5	n.d.	3.07 ± 0.91 a	0.96 ± 0.16 c	2.05 ± 0.90 a	0.82 ± 0.17 c	1.04 ± 0.11 b	6.90 ± 4.48 a	2.07 ± 1.18 bc	1.03 ± 0.06 b
84	n.d.	5.76 ± 1.81 a	1.58 ± 0.61 b	n.d.	1.71 ± 0.21 b	0.99 ± 0.14 b	n.d.	1.66 ± 0.25 b	0.95 ± 0.04 b
150	n.d.	n.d.	2.50 ± 0.68 b	n.d.	2.23 ± 1.05 ab	0.75 ± 0.12 bcd	n.d.	7.21 ± 0.43 a	0.85 ± 0.11 b
270	n.d.	n.d.	24.8 ± 2.12 a	n.d.	3.14 ± 1.39 a	1.62 ± 0.43 a	n.d.	n.d.	1.85 ± 0.57 a
	P. vulgaris			*R. sativus*			*H. annuus*		
0	0.06 ± 0.09	<0.06 b	<0.06 c	0.58 ± 0.11 c	0.28 ± 0.03 c	0.41 ± 0.12 c	0.32 ± 0.18 c	0.25 ± 0.05 e	0.21 ± 0.01 f
8	n.d.	0.92 ± 0.36 a	0.07 ± 0.03 c	4.00 ± 1.01 b	10.6 ± 1.57 b	0.63 ± 0.12 c	8.43 ± 1.06 b	1.80 ± 0.08 d	0.34 ± 0.07 f
14.5	n.d.	0.93 ± 0.20 a	0.27 ± 0.09 bc	8.83 ± 2.48 a	11.1 ± 0.60 b	1.09 ± 0.03 c	10.9 ± 0.84 ab	3.34 ± 0.98 c	0.43 ± 0.02 f
26	n.d.	1.72 ± 0.54 a	0.66 ± 0.31 ab	14.1 ± 1.71 a	10.3 ± 1.17 b	1.81 ± 1.04 c	13.9 ± 2.45 a	5.69 ± 0.68 b	0.79 ± 0.15 e
46.5	n.d.	n.d.	1.30 ± 0.65 a	n.d.	10.4 ± 0.40 b	6.11 ± 2.27 b	n.d.	10.8 ± 0.78 a	2.15 ± 0.33 d
84	n.d.	n.d.	n.d.	n.d.	18.8 ± 2.64 a	9.05 ± 2.38 ab	n.d.	12.5 ± 1.48 a	4.94 ± 0.67 c
150	n.d.	n.d.	n.d.	n.d.	n.d.	12.2 ± 3.40 ab	n.d.	n.d.	6.58 ± 0.35 b
270	n.d.	n.d.	n.d.	n.d.	n.d.	16.0 ± 3.12 a	n.d.	n.d.	9.70 ± 1.96 a

Means followed by the same letter in the same column and species did not significantly differ from each other (one-way ANOVA, Tukey test, $p > 0.05$). n.d. - not determined due to lack of biomass for analysis.

Table 7. Mean value (mean ± standard deviation, n = 3 to 5) of bioconcentration factor (ratio between plant content and total soil content) for arsenic in rice plants (*Oryza sativa*), maize (*Zea mays*), sorghum (*Sorghum bicolor*), beans (*Phaseolus vulgaris*), radish (*Raphanus sativus*) and sunflower (*Helianthus annuus*) cultivated in ATS, Oxisol and Inceptisol artificially contaminated by arsenic with increasing concentrations. Values are expressed in mg kg^{-1}.

	O. sativa			*Z. mays*			*S. bicolor*		
Dose	ATS	Oxisol	Inceptisol	ATS	Oxisol	Inceptisol	ATS	Oxisol	Inceptisol
8	0.71 ± 0.12 ab	0.31 ± 0.01 a	0.06 ± 0.01 ab	0.17 ± 0.01 a	0.13 ± 0.02 a	0.05 ± 0.01 ab	0.16 ± 0.01 a	0.17 ± 0.01 a	0.03 ± 0.01 bc
14.5	0.36 ± 0.10 bc	0.19 ± 0.02 b	0.10 ± 0.01 a	0.09 ± 0.00 b	0.06 ± 0.00 b	0.07 ± 0.02 a	0.10 ± 0.03 a	0.10 ± 0.00 b	0.06 ± 0.01 a
26	0.99 ± 0.00 a	0.14 ± 0.03 bc	0.04 ± 0.00 bc	0.04 ± 0.02 c	0.03 ± 0.01 bc	0.04 ± 0.00 bc	0.08 ± 0.01 a	0.06 ± 0.01 bc	0.04 ± 0.01 ab
46.5	n.d.	0.08 ± 0.02 bc	0.03 ± 0.01 bc	0.05 ± 0.02 bc	0.02 ± 0.00 c	0.03 ± 0.00 bcd	0.17 ± 0.11 a	0.05 ± 0.03 bc	0.03 ± 0.00 bc
84	n.d.	0.08 ± 0.03 c	0.02 ± 0.01 c	n.d.	0.02 ± 0.00 bc	0.01 ± 0.00 cd	n.d.	0.02 ± 0.00 c	0.01 ± 0.00 c
150	n.d.	n.d.	0.02 ± 0.01 bc	n.d.	0.02 ± 0.01 c	0.01 ± 0.00 cd	n.d.	0.05 ± 0.00 bc	0.01 ± 0.00 c
270	n.d.	n.d.	0.10 ± 0.01 a	n.d.	0.01 ± 0.01 c	0.01 ± 0.00 d	n.d.	n.d.	0.01 ± 0.00 c
	P. vulgaris			*R. sativus*			*H. annuus*		
8	n.d.	0.17 ± 0.06 a	0.01 ± 0.00 b	0.56 ± 0.14 a	1.90 ± 0.28 a	0.08 ± 0.02 bc	1.17 ± 0.15 a	0.32 ± 0.01 a	0.04 ± 0.01 cd
14.5	n.d.	0.08 ± 0.02 b	0.04 ± 0.01 ab	0.62 ± 0.17 a	0.92 ± 0.05 b	0.15 ± 0.00 abc	0.76 ± 0.06 b	0.28 ± 0.08 ab	0.06 ± 0.00 bc
26	n.d.	0.08 ± 0.02 ab	0.03 ± 0.01 ab	0.56 ± 0.07 a	0.47 ± 0.05 c	0.08 ± 0.05 bc	0.55 ± 0.10 b	0.26 ± 0.03 ab	0.03 ± 0.01 d
46.5	n.d.	n.d.	0.04 ± 0.02 a	n.d.	0.26 ± 0.01 cd	0.19 ± 0.07 ab	n.d.	0.27 ± 0.02 ab	0.07 ± 0.01 b
84	n.d.	n.d.	n.d.	n.d.	0.27 ± 0.04 cd	0.10 ± 0.03 bc	n.d.	0.18 ± 0.02 bc	0.06 ± 0.01 bc
150	n.d.	n.d.	n.d.	n.d.	n.d.	0.11 ± 0.03 bc	n.d.	n.d.	0.06 ± 0.00 bc
270	n.d.	n.d.	n.d.	n.d.	n.d.	0.06 ± 0.01 c	n.d.	n.d.	0.04 ± 0.01 d

Means followed by the same letter in the same column and species did not significantly differ from each other (one-way ANOVA, Tukey test, $p > 0.05$). n.d. - not determined due to lack of biomass for analysis.

4. Discussion

The percentage of the applied concentration obtained in the total As extraction was generally high (87.3% on average), moreover, chemical analyses confirm that the plants were exposed to an increasing gradient of As in all tested soils. Regarding the extractable contents, the concentrations and recovery rates were higher in ATS, followed by Oxisol and Inceptisol.

The pattern of availability observed in the present study (ATS>Oxisol>Inceptisol) is in agreement with the relative toxicities among the three tested soils. Overall, the higher the As content extractable, the higher the toxicity observed (lower EC_{50} values) and the higher the As concentrations measured in plant tissues.

The different As availabilities/toxicities observed in the tested soils are also related to the As adsorption capacity of each soil. Therefore, a higher As adsorption capacity (AAC) corresponded to a lower toxicity of this element to plants, which confirms once again the observed toxicity gradient (Tables 3 and 4). Although the AAC has not been measured for ATS, presumably it is lower than the AAC of natural soils given its lower iron oxides content (Fe_2O_3; Table 1). Considering the high affinity of As by these iron oxides/hydroxides (Dai et al., 2016; Garg and Singla, 2011; Melo et al., 2012; Wang et al., 2015; Zhao et al., 2009), it is expected that soils with a higher content of iron-rich minerals have lower As availability (Otero et al., 2016) and, accordingly, higher AAC and, therefore, lower toxicity. These data are supported by the results obtained in the study conducted by Kader et al. (2016), where EC_{50} was estimated for As using the dry weight of *Cucumis sativus* L. shoots grown in natural Australian soils during four weeks. The result of this study also stresses that As phytotoxicity is lower when the Fe oxides/hydroxides content in the soil is higher.

The importance of As availability in assessing plant toxicity can be observed by comparing the EC_{20} and EC_{50} values based on total and extractable As concentrations. While EC_{20} and EC_{50} values, based on the total As for the same species and using the same endpoint show considerable differences among soils, the corresponding values of EC_{20} and EC_{50}, based on extractable As, are considerably similar for different soil. This can also be seen in the work of Van Gestel et al. (2012). These authors assessed the toxicity of molybdenum to plants in spiked soil. The toxicity values to shoot yield in two contrasting soil, Zwijnaarde soil (Fe – dithionite: 1.40 g kg^{-1}, effective CEC: 4.10

339 $cmol_c\ kg^{-1}$; clay: 2.20 %) and Woburn soil (Fe – dithionite: 43.2 g kg^{-1}, effective CEC:
340 30.0 $cmol_c\ kg^{-1}$; clay: 31.4 %) did not have their confidence intervals overlap when
341 derived on total concentrations, but it usually occurred when soil solution concentration
342 were used. In another work conducted by Kader et al. (2017) this also occurred. This
343 fact not only highlights the influence of As availability on toxicity but also strengthen
344 that the use of extractable contents for the calculation of the EC_{20} or EC_{50} values allows
345 getting more realistic and comparable values among different soils, because it considers
346 only the available fraction of metals (Peijnenburg et al., 2007).
347 Although it is rare to find natural soils with attributes similar to those of artificial
348 tropical soil, their use for arsenic ecotoxicological studies is recommended. This is due
349 to great arsenic availability in this soil, which represent soils with less arsenic adoption
350 capacity more sensitive to arsenic toxicity.
351 The species *O. sativa*, *R. sativus*, and *H. annuus* were the ones that had the
352 greatest capacity to accumulate As in the tissues, presenting the highest As
353 concentrations in the shoots and higher bioconcentration factors. *Oriza sativa* has been
354 identified as one of the highest As accumulating plant among cereal species (Wang et
355 al. 2015). This species presented levels ranging from 0.78 to 24.9 mg kg^{-1} between the
356 treatments 14.5 and 46.5 mg kg^{-1} (Table 6) respectively, which is within the range of
357 0.89 to 21.9 mg kg^{-1} reported by Shrivastava et al. (2017) in plants of this species,
358 evaluated in different phenological stages collected from soils with As contents ranging
359 between 16.2 to 54.2 mg kg^{-1}.
360 In the case of *R. sativus*, the high As accumulation capacity observed in the
361 present study (up to a maximum of 18.8 mg kg^{-1}) is in agreement with the study
362 conducted by Smith; Koch; Reimer (2008), in which *R. sativus* plants were exposed in
363 hydroponic media for 31 days at increasing As doses of up to 50 mg L^{-1}. The As content
364 measured in the leaves reached 210 mg kg^{-1} (in dry matter) in the highest treatments.
365 Although this concentration is considerably higher than the concentrations measured in
366 the present study, the high As accumulation capacity by these plants is confirmed.
367 Regarding the *H. annuus* species, it has been considered a multiple metals
368 hyperaccumulating species (Cutright et al., 2010). Specifically for As, according to a
369 study conducted by January et al. (2008), *H. annuus* has a high capacity to accumulate
370 As in hydroponic media. Another study reported a higher As accumulation in *H. annuus*
371 than in *Z. mays* (Gulz; Gupta; Schulin 2005). However, other authors have still
372 considered this plant a non-hyperaccumulating As species (Shaheen and Rinklebe,

2015; Yadav et al., 2014) and As-nontolerant plant (Raab et al., 2005), which somehow contradicts the results observed in the present study.

On the other hand, the species *Z. mays*, *S. bicolor*, and *P. vulgaris* were the ones that presented lower As accumulation capacity in their tissues. The contents and bioconcentration factors of As found in *Z. mays* were close to those observed in previous research. Drličková et al. (2013) cultivated *Z. mays* for 15 weeks in Ocher soil with pH 4.7, As total of 237 mg kg^{-1} and As extractable (NH_4NO_3) of 22 mg kg^{-1} and in Heap soil with a pH of 4.2, As total of 90 mg kg^{-1} and As extractable (in NH_4NO_3) of 34 mg kg^{-1}. The As contents were 0.6 and 2.45 mg kg^{-1} and the bioconcentration factors were 0.003 and 0.027, for Ocher and Heap soil, respectively. In another study developed by Gulz et al. (2005), *Z. mays* was grown for four months on a silty loam soil (pH = 7.4, clay = 32% and silt = 50%) and in a sandy loam soil (pH = 7.2, clay = 18% and silt = 2%) containing 2.8 mg kg^{-1} of phytoavailable As (extracted by 0.1 M $NaNO_3$). At the end of the experiment, the plants presented As contents of 1.0 and 3.0 mg kg^{-1} in the silty loam soil and 7.0 and 9.0 mg kg^{-1} in the sandy loam soil, in stems and leaves, respectively. In another study developed by Silva et al. (2015), *Z. mays* plants were grown in nutrient solution containing 5 mg L^{-1} of As (68 μmol L^{-1}) for 21 days and the As contents observed in the shoots and leaves were 1.37 and 0.66 mg kg^{-1}, respectively. Despite the difference between the cultivation times, the average As content obtained for stems and leaves (shoot) observed for silty loam soil (Gulz et al., 2005) and nutrient solution (Silva et al., 2015) are close to the levels obtained for this crop in the Oxisol, the Inceptisol and the ATS.

The species *P. vulgaris* was the one that showed highest As sensitivity, generally presenting the lowest values of EC_{20} and EC_{50} for the two considered endpoints. The high sensitivity of *P. vulgaris* is also recognized by Aracil et al. (2001) and Lario et al. (2002). These authors exposed *P. vulgaris* to three doses of As (0.2, 0.5 end, 1.0 mg L^{-1}), in the form of methylarsonic acid and dimethylarsinic acid in hydroponic media for 28 days. These authors attributed the As sensitivity of *P. vulgaris*, among other factors, to its low capacity for biochemical detoxification. In another study conducted by Talukdar (2013), it was argued that one of the main causes of reduced growth of *P. vulgaris* exposed to As is the increase in the malondialdehyde content and loss of the photosynthetic apparatus function. Although the experiments reported were performed with organic As (Aracil et al., 2001; Lario et al., 2002) and in hydroponic media (Aracil et al., 2001; Lario et al., 2002; Talukdar, 2013), which makes comparison with the data

of the present study difficult, they provide an indication of the effect of inorganic As on *P. vulgaris*, since it is assumed that the forms of inorganic As (as the case of sodium arsenate used in the present study) are more toxic than forms of organic As (Aracil et al., 2001; Lario et al., 2002; Meharg and Hartley-Whitaker, 2002; Shri et al., 2009; Souza et al., 2015; Yoon et al., 2015).

The low As concentrations observed in the tissues of *Z. mays*, *S. bicolor*, and *P. vulgaris* species may be related to protection mechanisms, since it is known that non-hyperaccumulating plants tend to restrict the transfer of the contaminant to shoots (Shaheen and Rinklebe, 2015) but allowing higher accumulation in roots (Finnegan and Chen, 2012). However, it would be necessary to measure the As concentration in the roots of these plants in order to confirm this hypothesis.

Overall, As concentrations in plant tissues and the bioconcentration factors among the tested species do not appear to be related to the observed relative toxicities. While the species most sensitive to As (*P. vulgaris*) had low As concentrations in tissues and low BFs, the less sensitive species (*Z. mays*) was also found in the group of species that presented lower As concentrations in tissues and lower BFs.

The current prevention value for As (PVAs) in the State of Minas Gerais, Brazil, is 15 mg kg^{-1}. Since it is higher than all $HC5_{Total}$ values derived in ATS, the current Brazilian PV_{As} would affect in 50% the early germination count and production dry mass of more than 5% of the species in a soil with arsenic availability similar to ATS, but probably not more 50% of species. In Inceptisol, the PV_{As} probably would affect in less than 20% the early germination count and production dry mass of less than 5% species, because all $HC5_{Total}$ values derived are bigger than PV_{As}. On the other hand, in Oxisol, this is only true for $HC5_{Total}$ values of dry mass. Thus, the PV_{As} is not protecting in more than 50% the early germination count of more than 95% of species. Therefore, the PVAs can only be considered adequate for soils with high arsenic adsorption capacity (similar to Inceptisol). For all other situations, more investigations have necessary for confirm at PVAs adequacy, because in present study was showed it can be less protective for soils that have low adsorption capacity, in which available contents are close to total contents.

The HC values resulting from the present study should be seen as preliminary values, for greater robustness that must be complemented with data on sensitivity of more species using the same soils.

5. Conclusions

The species of plants tested showed different sensitivities to As and As concentrations in tissues and BFs were not related to the relative toxicities among the species to As. Among the studied species, *P. vulgaris* is the most sensitive species to As toxicity, and also the one that accumulated lower As contents in the tissues of shoots and presented lower BF values along with the species *Z. mays* and *S. bicolor*. In general, *Z. mays* was the least sensitive species and *H. annuus*, *O. Sativa*, and *R. sativus* were the species that accumulated more As and had the highest BFs.

The use of extractable concentrations in As toxicity characterization, as opposed to total contents, allowed to estimate more realistic and comparable toxicity values (EC_{20} and EC_{50}) among different soils. Thus, the results suggest that in the future, the definition of prevention values for metals shall be performed based on extractable contents.

In the present study, the current Brazilian prevention value for arsenic is adequate for soils with high arsenic adsorption capacity.

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ARTIGO 3 Toxicity of arsenic to soil organisms and its relation with the current soil screening value for arsenic (prevention value) in soils from Minas Gerais, Brazil

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Toxicity of arsenic to soil organisms and its relation with the current soil screening value for arsenic (prevention value) in soils from Minas Gerais, Brazil

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Abstract

Soil screening values (e.g., prevention values) established by law for many soil contaminants – including arsenic (As) – are important tools to manage contaminated areas, and, therefore, the adequacy of these values should be evaluated. The objective of this study was to assess As toxicity to soil invertebrate species and to verify the protection level of the As prevention value adopted by Minas Gerais State, Brazil. In order to accomplish this, the effect of As on reproduction and survival of the species *Eisenia andrei*, *Enchytraeus crypticus*, *Folsomia candida*, and *Hypoaspis aculeifer* was investigated exposing these species in two Brazilian natural soils (Red-yellow Latosol - Oxisol, Haplic Cambisol - Inceptisol) and one artificial tropical soil (ATS) spiked with increasing As concentrations (0; 8; 14.5; 26; 46.5; 84; 150; 270 mg kg^{-1}). The highest As toxicity was found in the ATS, in which *E. crypticus* was the most sensitive species and *H. aculeifer* was the most resistant. EC_{50} reproduction values (based on total As concentration) varied from 20.5 to 98.4 mg kg^{-1} in the ATS, 62.0 to >271 mg kg^{-1} in the Oxisol, and >232 mg kg^{-1} in the Inceptisol. Results obtained in this study demonstrated that the current prevention value in Minas Gerais State for arsenic is protective for soils with high adsorption capacity and probably not for soil with lower arsenic adsorption capacity.

Keywords: arsenate, tropical soils, environmental quality, contamination, threshold concentration.

1. Introduction

Soils are the basis of several key ecological services (Faber and van Wensem, 2012), but increasing soil pollution can compromise the health of terrestrial systems and, consequently, its functionality. Aiming to control and support the management of soil contamination, prevention values have been established by law in Brazil for several contaminants (CONAMA, 2009). The prevention values established by the Brazilian legislation are soil screening values that refer to the content of specific elements/substances in the soil above which soil functionality can be compromised (CONAMA, 2009). Soil function is defined as the capacity to serve as a medium for life and habitat sustainability humans, animals, plants and other living organisms.

Arsenic (As) is one of such soil contaminants. It is a broadly dispersed metalloid and may have its contents increased in the soil by natural processes (e.g., weathering of As-containing rocks) or by anthropogenic activities (e.g., disposal of industrial residues, burning of fossil fuels, and mining exploitation) (Mandal and Suzuki, 2002). Arsenic soil contamination constitutes a serious concern not only due to its effects on human health - as a carcinogenic element -, but also due to its known noxious effects on soil biota (Kabata-Pendias, 2011; Kabata-Pendias and Mukherjee, 2007).

In Brazil, there are different soil classes that present distinct chemical and physical attributes (Santos et al., 2013). The soil attributes (e.g. texture, organic matter, pH, mineralogy) are known to influence the availability of contaminants, and consequently, their toxicity to the biota (Kader et al., 2016; Lanno et al., 2004; Lee et al., 2013; Romero-Freire et al., 2015, 2014). Therefore, chemical quantification of total metals in soil is an important requirement for soil quality evaluation, but it is not sufficient to evaluate the environmental risk of contaminated sites (Cesar et al., 2015; González et al., 2011). In addition, different organisms present distinct sensitivities and contaminant exposure pathways, which leads to the necessity of using several organisms, representative of the panoply of exposition modes present in terrestrial systems, during the evaluation of substance toxicity (Romero-Freire et al., 2015).

The goal of this study was to evaluate As toxicity to different soil organisms and in three different soils, in order to verify the adequacy of the protection level for the adopted As prevention value in Minas Gerais State, Brazil. For that, two natural soils from Minas Gerais (an Oxisol and an Inceptisol) and an artificial tropical soil were spiked with increasing doses of sodium arsenate and used in ecotoxicological tests. This

study complements a series of laboratory ecotoxicological tests and a recent publication presented data obtained with plant growth tests (Martins et al. *in prep*.). Our findings present and discuss data obtained in laboratory tests with the standard invertebrate species: *Eisenia andrei*, *Enchytraeus crypticus*, *Folsomia candida*, and *Hypoaspis aculeifer* and the protection level for As provided by the current prevention value established in the Brazilian legislation.

2. Materials and methods

2.1 Soils

Three soils were used in laboratory experiments: Red-yellow Latosol (Oxisol) collected in the municipality of Itumirim, Minas Gerais, Brazil (Latitude E-21°17'08'', Longitude N-44°47'43''), Haplic Cambisol (Inceptisol) collected in the municipality of Lavras, Minas Gerais, Brazil (Latitude E-21°13'46, Longitude N-44°59'10), and an artificial tropical soil (ATS), produced by a mixture of kaolinite clay, fine sand, and coconut fiber in a proportion of 2:7:1 (in a dry weight basis). Both natural soils were collected at 0–20-cm depth, and air dried and sieved at 2 mm before their use. For each soil, texture, pH, cation exchange capacity at pH 7 (CEC), organic matter content (OM), and remaining phosphorous analyses (P-Rem) were determined following procedures described by EMBRAPA (1997). Maximum water holding capacity (WHC) was measured according to ISO (ISO, 2011). In addition, As maximum soil adsorption capacity was determined for the natural soils following a methodology described by Campos et al. (2007). These data are presented in Table 1.

Natural soils were defaunated in two freezing-thawing cycles (48 h at -20ºC followed by 48 h at 25ºC for each cycle) and then employed in laboratory tests.

Table 1. Physicochemical attributes for soils used in ecotoxicological tests. Data from Martins et al. 2018, *submitted*.

Soil	Texture			pH (H_2O)	CEC	OM	P-rem	AAC	WHC	Fe_2O_3
	Clay	Silt	Sand							
	-------- % --------				*$cmol_c\ dm^{-3}$*	*%*	*$mg\ L^{-1}$*	*$mg\ kg^{-1}$*	*%*	*%*
ATS	19	8	73	5.2	2.27	7.84	36.1	n.d.	74.0 ± 0.02	1.80 ± 0.10
Oxisol	26	8	66	4.4	0.27	0.24	6.84	714.3	41.0 ± 0.01	2.40 ± 0.00
Inceptisol	33	48	19	4.6	2.01	1.87	4.31	1,667	59.0 ± 0.01	17.4 ± 1.03

Note: CEC – Cation exchange capacity at pH 7; OM – Organic matter; P-rem – remaining phosphorus; AAC – Maximum arsenic adsorption capacity; WHC – Maximum water hold capacity. Fe_2O_3 – Iron oxides. n.d. = not determined.

2.2 Test organisms and culture conditions

The test organisms used in the laboratory experiments were the predatory mite *Hypoaspis aculeifer* G. Canestrini (Laelapidae), the collembolan *Folsomia candida* Willem (Isotomidae), and the oligochaetes *Enchytraeus crypticus* Westheide & Graefe (Enchytraeidae) and *Eisenia andrei* Bouché (Lumbricidae).

All test organisms were obtained from cultures of the Soil Ecology and Ecotoxicology Laboratory of the Department of Life Sciences of the University of Coimbra, Portugal.

Mites, *H. aculeifer*, were maintained in boxes filled with a ~1 cm layer of a mixture of activated charcoal and plaster of Paris in a 1:9 ratio (w:w) and fed twice per week with *Tyrophagus putrescentiae*. Collembolans, *F. candida*, were kept in boxes with a layer of nearly 1 cm containing a mixture of activated charcoal and plaster of Paris in a 1:11 ratio (w:w) and fed once a week with granulated dry yeast. Enchytraeids, *E. crypticus*, were kept in petri dishes filled with solid bacteriological agar prepared according to César et al. (2015) and weekly fed with autoclaved ground oat. Earthworms, *E. andrei*, were maintained in boxes containing a mixture of peat, fresh cow manure (previously defaunated through freezing-thawing cycles) and water at a 4:4:3 ratio (w:w:w), adjusted to a pH of 7 through the addition calcium carbonate. Worms were weekly fed with fresh cow manure. All cultures and tests were kept at 25 ± 2ºC with a 16:8h photoperiod (light:dark).

122 2.3 Experimental procedure
123
124 The tested soils were contaminated in the laboratory with different volumes of a
125 heptahydrate sodium arsenate stock solution (Na_2HAsO_4 $7H_2O$, purity ≥ 98%, Sigma-
126 Aldrich, St. Louis, MO, USA) in order to reach a nominal gradient As concentration of:
127 0, 8, 14.5, 26, 46.5, 84, 150, 270 mg of As kg^{-1} of dry soil. The solution volume applied
128 in each soil was diluted in water to raise soil humidity to nearly 50% of its maximum
129 water holding capacity. Solutions were well mixed to the soil to obtain a homogenous
130 matrix. The same gradient of As concentrations was prepared for all tested soils and
131 used in all laboratory tests. Additional replicates were prepared for each test, using the
132 same soils with different concentrations of sodium chloride (NaCl, purity 99.7%,
133 JMGS, Odivelas, Portugal), as a salt control. In these replicates, salt concentrations
134 were used in order to simulate the ionic strength expected in As doses of 150 and 270
135 mg kg^{-1}.
136 Soil samples of all doses were collected from each laboratory test for chemical
137 analyses. Each sample was oven dried at 40°C until constant weight, grounded in an
138 agate mortar and sieved at 150 μm in a nylon sieve and submitted to a microwave
139 digestion with HNO_3, according to USEPA 3051A (USEPA, 2007), to determine semi-
140 total As concentrations (named total in this work). Arsenic available fraction of soil
141 samples was also measured through an extraction based on the mixture of 0.5 g of soil
142 sample with 5 mL of Mehlich-1 solution (0.05 mol L^{-1} HCl + 0.0125 mol L^{-1} H_2SO_4).
143 This mixture was shaken during 5 minutes and filtered through a 0.45-μm filter, after a
144 16-hour resting period (EMBRAPA, 1997). Arsenic concentrations in the total and
145 available extracts were determined by atomic absorption spectrophotometry with
146 electrothermal atomization using a graphite furnace (Perkin Elmer – AAnalyst™ 800).
147 In order to verify the accuracy of total content determinations, SRM 2710a
148 (Montana I soil) and SRM 2711a (Montana II soil) certified reference materials were
149 employed and an average As recovery of 101 ± 8.14% was obtained. Blanks were used
150 to determine the detection and quantification limits of 0.10 and 0.50 mg kg^{-1} for total
151 measurements and 0.03 and 0.09 mg kg^{-1} for available measurements, respectively
152 (Penha et al., 2017).
153 Laboratory reproduction tests followed standard guidelines from ISO and OECD
154 and the adopted details are summarized in Table 2.

Table 2. Procedures adopted in laboratory reproduction tests with *Hypoaspis aculeifer*, *Folsomia candida*, *Enchytraeus crypticus* and *Eisenia andrei*.

	H. aculeifer	*F. candida*	*E. crypticus*	*E. andrei*
Standard guideline followed	OECD 226 (2016)	ISO 11267 (1999)	ISO 16387 (2003)	ISO 11268-2 (2011)
Test period (days)	14	28	28	56
Test containers (cm)	4.5 x 10 (*D* x *H*)	4.5 x 10 (*D* x *H*)	4.5 x 10 (*D* x *H*)	10 x 16 (*D* x *H*)
Nº of organisms per replicate	10[a]	10[c]	10[d]	10[e]
Nº of replicates per control	9 + 1[b]	5 + 1[b]	9 + 1[b]	4
Nº of replicates per treatment	5 + 1[b]	5 + 1[b]	5 + 1[b]	4
Food source	*T. putrescentiae*	Dry yeast	Rolled oats	Cow manure
Food per test container (g FW)	a tip of a spatula	0.002	0.001	15
Days of food supply	0, 3rd, 7th, 10th	0, 14th	0, 7th, 14th, 21th	0, 14th, 28th
Days of aeration and moisture reestablishment	3rd, 7th, 10th	7th, 14th, 21th	7th, 14th, 21th	7th, 14th, 21st, 28th, 35th, 42nd, 49th
Soil per test container (g DW)	20	~30	20	500

D diameter, *H* height; [a] - *H. aculeifer* females aged between 28 and 35 days; [b] - Additional replicate without organisms to control soil pH and moisture content at the end of the test; [c] – *F. candida* aged between 10 and 12 days; [d] – *E. crypticus* with a similar size and a well-developed clitellum; [e] – *E. andrei* with less than 12 months of age, previously acclimatized in the test soil, individually weighted (weights between 250-600 mg) and with a well-developed clitellum.

In the reproduction tests with *H. aculeifer*, at the end of the test period, mites of each replicate were extracted with a 70% ethanol solution using a Macfadyen high-gradient extractor and employing temperature cycles suggested by OECD (2016). Afterwards, the number of mites per replicate was determined through a stereomicroscope.

In *F. candida* reproduction tests, at the end of the test period, the whole content of each test vessels was transferred to a larger container, which was imediately filled with water in a volume sufficient to submerge all soil, and then some drops of blue or black ink were added to increase the contrast between the water surface and the collembolans at floating water surface. After carefully stirring the vessels bottom with a spatule, the surface was photographed with a digital camera and the organisms were counted with the ImageJ software (Schneider et al., 2012).

At the end of the *E. crypticus* reproduction tests, organisms of each replicate were killed and fixed in 80% ethanol and stained with Bengal rose (1% in ethanol) over night. Subsequently, each replicate was washed in a 104-µm mesh sieve and enchytraeids were counted with a stereomicroscope.

In the reproduction tests with *E. andrei*, at the 28^{th} day of the test, surviving adults were removed from test containers, counted, washed and weighted to determine changes in body mass. After removing adults, the test containers with the cocoons that remained in the incubation chamber for an additional four-week period, after which the test vessels were placed in a water bath between 50 and 60ºC to extract and count manually the juveniles of each replicate (ISO, 2011).

2.4 Statistical analysis

Differences between treatments were tested by one-way ANOVA analysis followed by Dunnett post-hoc test to evaluate the differences between treatments and the control. Normality and homogeneity assumptions of data were previously checked through the Kolmogorov-Smirnov and Bartlett tests, respectively. In cases where these assumptions were not fulfilled, data were transformed by log10(x+1) or square root. EC_{50} and EC_{20} values (concentrations that reduce reproduction by 50 and 20%, respectively, when compared with the control) were estimated considering total and available As concentrations and through non-linear regressions using exponential, Gompertz, Hormesis or logistic models. These analyzes were accomplished using the

195 STATISTICA 7.0 software. LC_{50} values (concentrations that reduce survival by 50%
196 comparing with control) were estimated through Probit analysis using the PriProbit 1.63
197 software (Sakuma, 1998). For each test soil, the EC_{50} and EC_{20} values were used to
198 build a species sensitivity distribution curve (SSD) to estimate hazard concentration
199 values with 50 and 5% of protection level (HC_{50} and HC_5, respectively). These
200 calculations were performed using the ETX 2.0 software (Vlaardingen et al., 2004).
201
202 3. Results
203
204 The total concentrations of As in pure soils (controls) were between 1.68 and
205 3.10 mg kg^{-1}, and the available concentrations were always < 0.37 mg kg^{-1} in all tested
206 soils. Total As concentrations corresponded to recovery percentages were always higher
207 or equal to 71%, and this percentage was greater in the lowest nominal concentration
208 (Table 3). The available concentrations measured and the respective percentages of
209 recovery were higher in ATS, followed by Oxisol and Inceptisol (Table 3).
210

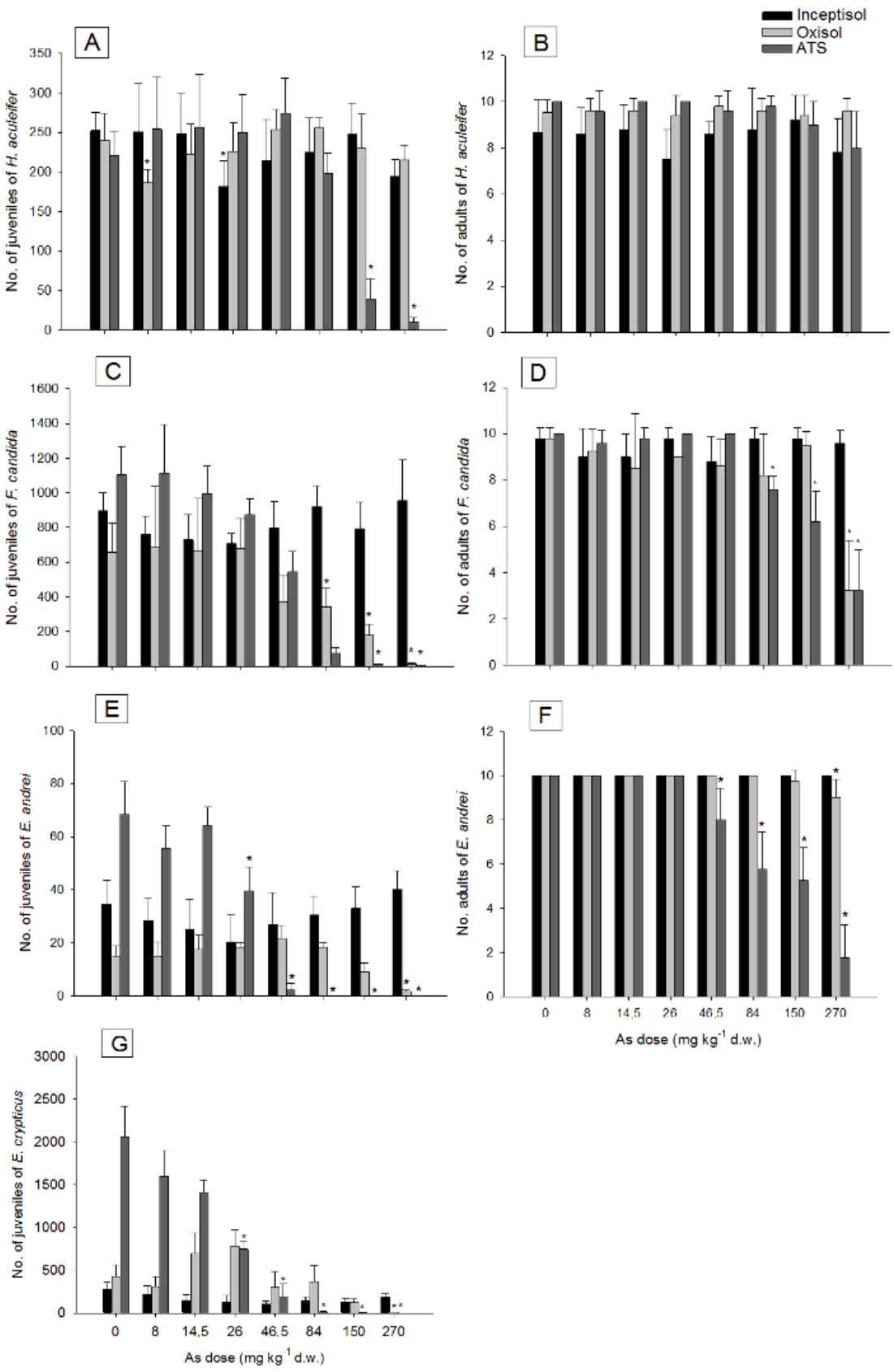
211 Table 3. Average concentrations (± standard deviation) of total (USEPA 3051A) and
212 available (Mehlich-1) arsenic, expressed in mg kg^{-1} and respective average of recovery
213 percentages (± standard deviation) with respect to the nominal concentrations in the
214 artificial tropical soil (ATS), the Oxisol, and the Inceptisol, used in laboratory
215 ecotoxicological tests standard soil invertebrates.
216

	Nominal mg kg^{-1}	Total mg kg^{-1}	% of recovery	Available mg kg^{-1}	% of recovery
ATS	0	3.10 ± 0.26	-	0.33 ± 0.09	-
	8	10.1 ± 1.21	126 ± 15.1	3.81 ± 0.63	47.6 ± 7.92
	14.5	16.1 ± 0.82	111 ± 5.65	5.92 ± 0.20	40.8 ± 1.37
	26	29.3 ± 0.30	113 ± 1.17	12.6 ± 1.09	48.5 ± 4.18
	46.5	43.5 ± 2.53	93.6 ± 5.43	23.1 ± 1.32	50.6 ± 2.89
	84	77.1 ± 0.77	91.8 ± 0.92	41.9 ± 3.46	49.9 ± 4.12
	150	131 ± 39.9	87.2 ± 26.6	98.2 ± 13.9	65. 4 ± 9.29
	270	259 ± 26.3	95.9 ± 9.75	208 ± 22.9	76.9 ± 8.48
Oxisol	0	2.43 ± 0.30	-	0.37 ± 0.07	-
	8	9.69 ± 3.47	121 ± 43.4	1.30 ± 0.77	16.2 ± 9.57
	14.5	14.5 ± 0.72	100 ± 4.95	2.00 ± 0.52	13.7 ± 3.59
	26	18.5 ± 2.67	71.0 ± 10.3	2.66 ± 0.43	10.2 ± 1.65
	46.5	40.9 ± 6.34	90.0 ± 13.6	5.48 ± 0.64	12.0 ± 1.41
	84	76.6 ± 6.01	91.2 ± 7.15	13.1 ± 2.95	15.6 ± 3.51
	150	140 ± 26.1	93.4 ± 17.4	25.5 ± 1.19	17.0 ± 0.79
	270	271 ± 43.5	100 ± 16.1	54.4 ± 6.59	20.1 ± 2.44
Inceptisol	0	1.68 ± 1.09	-	0.21 ± 0.08	-
	8	10.6 ± 3.17	132 ± 39.5	0.71 ± 0.12	8.96 ± 1.55
	14.5	20.1 ± 1.39	138 ± 9.60	0.42 ± 0.09	2.94 ± 0.63
	26	23.9 ± 1.34	92.0 ± 5.15	0.68 ± 0.09	2.62 ± 0.36
	46.5	36.5 ± 15.7	78.5 ± 33.8	1.23 ± 0.13	2.69 ± 0.29
	84	64.4 ± 3.80	76.7 ± 4.53	2.60 ± 0.20	3.10 ± 0.24
	150	140 ± 8.90	93.8 ± 5.93	4.92 ± 0.87	3.28 ± 0.58
	270	232 ± 37.1	85.9 ± 13.7	11.0 ± 1.41	4.06 ± 0.52

217

In reproduction tests with mites, collembolans and enchytraeids, all validity criteria defined in respective followed protocols were fulfilled. In laboratory tests with *E. andrei*, the validity criteria were also fulfilled, except for the test in Oxisol, where no more than 30 juveniles were found per control replicate. Despite this fact, the test with the Oxisol was considered valid since the quality of test organisms was confirmed in the test control replicates with ATS, in which the same batch of worms was used and more than 60 juveniles were found in average per each replicate of control. Replicates of salt controls (Table 2, data enclosed) did not show any significant effect compared with controls (uncontaminated soil).

In tests with gradients of As contamination, mite survival was not significantly affected (Figure 1 - B). Significant decreases in reproduction were observed in doses 8 and 26 mg kg^{-1} in the Oxisol and the Inceptisol, respectively. In ATS, reproduction was not affected until the 150 mg kg^{-1} dose (Figure 1 - A). Collembolans reproduction and survival were not effect in Inceptisol, but in ATS and in Oxisol, collembolan survival significantly decreased following the exposure from 84 and 270 mg kg^{-1}, respectively, while reproduction reduced at the 84 mg kg^{-1} dose for both soils (Figure 1 – C and D). Enchytraeids survival could not be measured in any test since some juveniles presented sizes similar to that of adults at the end of the test. Reproduction of enchytraeids was not affected in Inceptisol, but significanlty decreased at 270 mg kg^{-1} dose in Oxisol (Figure 1 – G). A significant decrease was observed at the 26 mg kg^{-1} dose for ATS. Reproductive output was considerably higher in controls of ATS compared with those of Inceptisol and Oxisol (Figure 1).



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242 Figure 1. Average reproduction (bars and left axis, ± standard deviation, *n* = 5 to 9) of
243 *Enchytraeus crypticus* (G), and average reproduction (left, ± standard deviation, *n* = 4 to

9) and survival (right, respectively, ± standard deviation, n = 4 to 9) of *Eisenia andrei* (E and F), *Folsomia candida* (C and D), and *Hypoaspis aculeifer* (A and B) when exposed to an Oxisol, an Inceptisol and an artificial tropical soil (ATS) artificially contaminated with increasing concentrations of arsenic. * - treatments that significantly differ from control by Dunnett's post hoc test ($p \leq 0.05$).

The percentage of initial biomass of surviving adults of *E. andrei* only significantly increased in the 46.5 mg kg^{-1} dose in Inceptisol (12% of increase). On the other hand, the reductions following the exposure from the 150 mg kg^{-1} dose in ATS, with losses always < 30% (Figure 1, data enclosed).

Survival and reproduction of *E. andrei* were not affected in Inceptisol, but survival was significantly affected at the 270 mg kg^{-1} dose and at the 46.5 mg kg^{-1} dose, whereas reproduction decreased at the 270 mg kg^{-1} dose and at the 26 mg kg^{-1} dose in Oxisol and ATS, respectively. Surviving adults found in treatments of ATS presented yellowish marks in the skin and lethargic movements starting at the 84 mg kg^{-1} dose. As observed for enchytraeids, reproduction of *E. andrei* was considerably higher in controls of ATS compared with those of Oxisol and Inceptisol (Figure 1 – E and F).

Toxicity endpoints estimated for each test organisms and test soil are presented in Tables 4 and 5. In general, the toxic values estimated from data obtained in tests with Inceptisol were higher than the highest tested concentration (232 mg kg^{-1}). Because of that, the SSD curve and the respective HC values derivation could not be estimated for the Inceptisol.

Table 4. LC_{50} (Lethal concentration to 50%) data for the effects on the survival (with respective 95% confidence intervals) and EC_{20} and EC_{50} (effect concentration to 20 and 50%) data for the effects on reproduction (with respective 95% confidence intervals) of *Hypoaspis aculeifer, Folsomia candida, Enchytraeus crypticus* and *Eisenia andrei* after exposed to increasing arsenic contamination in the natural soils - Oxisol and Inceptisol - and in an artificial tropical soil (ATS). The determined values were based on total As concentrations and expressed in mg As kg^{-1} dry soil.

Soil type	Test species	LC_{50}	EC_{20}	EC_{50}
ATS	*H. aculeifer*	> 259	81.2 (72.0 - 90.4)	98.4 (86.4 - 110)
	F. candida	179 [(a)]	27.1 (20.1 - 34.1)	43.4 (37.8 - 49.0)
	E. crypticus	n.d.	10.7 (6.27 - 15.2)	20.5 (15.6 - 25.3)
	E. andrei	114 (96.3 - 139)	24.2 (18.1 - 30.3)	31.0 (27.1 - 34.9)
Oxisol	*H. aculeifer*	> 271	> 271	> 271
	F. candida	> 271	26.6 (0.74 - 52.5)	62.0 (29.4 - 94.7)
	E. crypticus	n.d.	54.0 (1.16 - 106)	90.9 (39.4 - 142)
	E. andrei	> 271	111 (58.5 - 164)	142 (117 - 166)
Inceptisol	*H. aculeifer*	> 232	> 232	> 232
	F. candida	> 232	> 232	> 232
	E. crypticus	n.d.	> 232	> 232
	E. andrei	> 232	> 232	> 232

[a] – Data does not allow estimation of a 95% confidence interval; n.d. = not determined since was not possible to separate adults from juveniles.

Table 5. LC_{50} (Lethal concentration of 50%) data for the effects on the survival (with respective 95% confidence intervals) and EC_{20} and EC_{50} (effect concentration of 20 and 50%) data for the effects on reproduction (with respective 95% confidence intervals) of *Hypoaspis aculeifer*, *Folsomia candida*, *Enchytraeus crypticus*, and *Eisenia andrei* after exposed to gradients of increasing arsenic contamination in the natural soils - Oxisol and Inceptisol - and in an artificial tropical soil (ATS). The determined values were based on available As concentrations and expressed in mg As kg^{-1} dry soil.

Soil type	Test species	LC_{50}	EC_{20}	EC_{50}
ATS	*H. aculeifer*	> 208	45.2 (37.8 - 52.7)	61.2 (49.8 - 72.7)
	F. candida	135 [(a)]	12.7 (8.55 - 16.8)	22.3 (18.7 - 25.8)
	E. crypticus	n.d.	3.69 (2.14 - 5.25)	8.26 (6.18 - 10.3)
	E. andrei	75.9 (60.9 - 98.2)	9.39 (5.76 - 13.2)	13.6 (11.0 - 16.3)
Oxisol	*H. aculeifer*	> 54.4	> 54.4	> 54.4
	F. candida	> 54.4	n.e.	9.78 (3.13 - 16.4)
	E. crypticus	n.d.	n.e.	16.5 (6.13 - 26.8)
	E. andrei	> 54.4	19.6 (9.30 - 30.0)	25.9 (20.9 - 30.9)
Inceptisol	*H. aculeifer*	> 11.0	> 11.0	> 11.0
	F. candida	> 11.0	> 11.0	> 11.0
	E. crypticus	n.d.	> 11.0	> 11.0
	E. andrei	> 11.0	> 11.0	> 11.0

n.e. = Data does not allow estimation.

The SSD curves and the HC_5 and HC_{50} values for soils ATS and Oxisol are presented in Figure 2 and Table 6. The *H. aculeifer* was the most resistant species in all tested soils, while *E. crypticus* and *F. candida* were the most sensitive species in ATS and Oxisol, respectively.

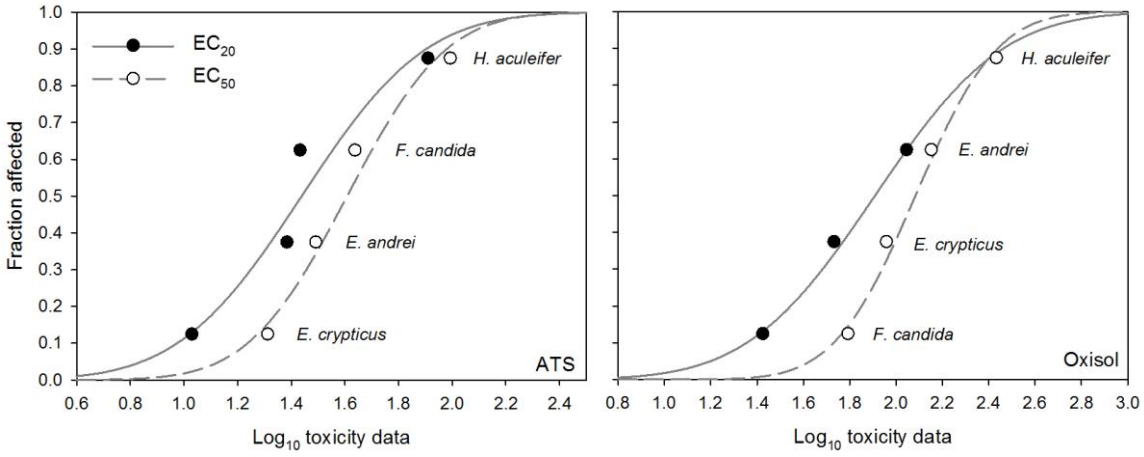

Figure 2. Species sensitivity distribution curves based on EC_{20} (first line of graphs) and EC_{50} values (second line of graphs) estimated through data of reproduction of the species *Hypoaspis aculeifer*, *Folsomia candida*, *Enchytraeus crypticus* and *Eisenia andrei* when exposed to gradients of increasing concentrations of arsenic in an artificial tropical soil (ATS – left) and an Oxisol (right). Note: The species at the bottom of Figure 2 were considerate more sensitive than those at the top.

Table 6. Comparison between the current prevention value (PV_{As}) considered for arsenic in Minas Gerais state, Brazil (CONAMA, 2009) and the hazardous concentration (HC) values at 5 and 50% (HC_5 and HC_{50}, respectively) estimated from EC_{20} and EC_{50} values (based on total and available As concentration) using data of reproduction of the species *Hypoaspis aculeifer*, *Folsomia candida*, *Enchytraeus crypticus* and *Eisenia andrei* when exposed to gradients of increasing arsenic concentrations in an artificial tropical soil (ATS) and an Oxisol. Values are expressed in mg of As kg^{-1} of dry soil.

		ATS		Oxisol	
		Total	Available	Total	Available
HC_5	EC_{20}	5.98 (0.37 - 14.5)	1.78 (0.05 - 5.50)	13.1 (0.48 - 38.7)	n.d.
	EC_{50}	12.0 (1.32 - 24.7)	4.14 (0.24 - 10.4)	38.0 (4.66 - 75.7)	5.77 (0.51 - 12.7)
HC_{50}	EC_{20}	27.4 (10.3 - 73.1)	11.8 (3.51 - 40.1)	81.0 (25.1 - 261)	n.d.
	EC_{50}	40.5 (18.5 - 88.7)	19.7 (7.23 - 54.1)	121 (57.5 - 255)	21.8 (9.28 - 51.3)
PV_{As}		15			

n.d. = Not determined.

4. Discussion

In general, independently of possible influences of soil properties, the total As concentrations measured corroborate whit nominal concentrations from tested treatments in all soils.

Concerning the available As concentrations measured, there is a clear indication that the available fraction of As is quite related to soil type. The highest availability of As was measured in treatments applied to ATS, followed by Oxisol and Inceptisol. It is expected that the smaller the As adsorption capacity, the higher As availability, and consequently, the higher As toxicity. In fact this assumption was confirmed by our data.

In general, the toxicity of As followed the sequence ATS>Oxisol>Inceptisol. This also matches the pattern observed for the P-rem values (Table 1). Higher P-rem values indicate a smaller phosphorous retention capacity. This fact is related to the high chemical similarity between phosphate and arsenate ions, which makes these two elements to compete for the same sorption sites (Cullen and Reimer, 1989; Kabata-Pendias, 2011; Smith et al., 1998). Thus, it is expected that soils with higher P-rem values present a smaller As adsorption capacity and, consequently, higher As availability and toxicity.

Soil mineralogy is another factor influencing As soil retention capacity. It is known that As has high affinity for iron oxides/hydroxides (Dai et al., 2016; Garg and Singla, 2011; Kabata-Pendias, 2011; Melo et al., 2012; Wang et al., 2015; Zhao et al., 2009). The natural soils used in the present study have more iron oxides than the ATS (Table 1), which explains the higher availability of As found in ATS compared with that of the natural soils. Our data agrees with the study conducted by Alves and Rietzler (2015), who verified a higher sensitivity of *E. andrei* to As in ATS, when compared with a natural soil rich in iron oxides.

In general, the less sensitive species used in laboratory experiments was *H. aculeifer*. The significant decreased reproduction found in the doses 8 and 26 mg kg^{-1} of Oxisol and Inceptisol, respectively, do not appear to be related to the As contamination since no negative effects were found in the subsequent higher concentrations. The high resistance of mites found in the present study agrees with several studies that have evidenced low sensitivity of *H. aculeifer* to other chemicals and even residues, compared with collembolans, enchytraeids, and earthworms (Huguier et al., 2015; Owojori et al., 2014; Pereira et al., 2015; Renaud et al., 2017). Assuming that the main

contaminant absorption pathways for mites are ingestion of soil solution and direct contact of the contaminant with permeable parts (leg articulations) (Huguier et al., 2015), In the laboratory tests performed in the present study, feeding did not represent a contribution for exposure of the organism to the contaminant, since the weekly supply of cheese mites (*Tyrophagus putrescentiae*) to the experimental vessels made the available food supply always fresh, and consequently free of contamination. By this way, the adopted procedure excluded eventual effects caused by contamination of preys, which could somehow contribute to an underestimation of the effect of the contaminant over these organisms. However, this assumption still needs to be confirmed with further investigation.

Regarding collembolans, *F. candida* species was the most sensitive to As in the Oxisol, but it was the least sensitive after mites in ATS, and not sensitive in Inceptisol, as happened with other test species. Since collembolans are hard body organisms, their As exposure pathways are absorption of soil solution through specialized organs and food ingestion (Peijnenburg et al., 2012; Vijver et al., 2004). Differently to what happened with mites, in the tests performed, food ingestion contributes for exposure of *F. candida* to As since the yeast supplied as food has the capacity to rapidly absorb the contaminant from the soil solution. Besides, collembolans can ingest As-contaminated organic matter directly from the soil. Despite that, the reproduction EC_{50} value estimated for ATS (43.4 mg kg^{-1}) was higher than the EC_{50} value of 2.19 mg kg^{-1} reported by Crouau and Cazes (2005) and Crouau and Moïa (2006), who exposed *F. candida* individuals to an artificial OECD soil (quartz sand: 70%, kaolinite clay: 20%, and peat: 10%) contaminated with As. The different observed sensitivities can be related to the distinct OM quality used in the OECD soil and in ATS (peat *vs* coconut fiber, respectively), to the test temperature employed (20±1ºC *vs* 25±2ºC, respectively; at 25ºC individuals of *F. candida* with 10 to 12 days of age have higher size than at 20ºC, which could make them more resistant to As), and to different exposure times used (35 days *vs* 28 days, respectively, even though an increase of exposure time can lead to a reduction in the LC_{50} value (Vijver et al., 2004) it is expectable that a larger exposure time leads to a reduction of the EC_{50} values).

Concerning both oligochaete species, the higher reproductive outcome observed in controls of ATS compared with controls of natural soils might be explained by the soil properties. It is known that these species prefer soils with pH of 5 to 6 and organic matter content higher than 3% (Jänsch et al., 2005; Natal-da-Luz et al., 2008).

Additionally, in general, these organisms prefer coarse soils with less clay, since a higher clay content can cause more stress due to the high energy consumption for movement (González-Alcaraz and van Gestel, 2016). These soil properties can explain the higher reproduction observed in ATS, which was the soil with the coarser texture (19, 8 and 73 % of clay, silt and sand, respectively) and higher organic matter content (7.8%).

Despite that, from all three tested soils, only Oxisol did not fulfill the criterion of 30 juveniles by control replicate at the end of the reproduction tests with *E. andrei*. This low reproduction could have been caused by low organic matter content (0.24%, the lowest OM among the three tested soils). It is known that reproduction of *E. andrei* often decreases in soils poor in OM content (Jänsch et al., 2005; Romero-Freire et al., 2015; Santorufo et al., 2012).

Belonging to the oligochaetes, *E. andrei* and *E. crypticus* have similar routes of exposure to contaminants, which are essentially through dermal, intestinal and respiratory contact and due to the direct contact with particles and soil solution (Castro-Ferreira et al., 2012). In the present study, both species were sensitive to the gradients of As contamination, generally showing a median sensitivity to As compared with other test species, except for ATS, where *E. crypticus* was the most sensitive. The sensitivity of *E. crypticus* to As contamination corroborates the data reported by Vašíčková *et al.* (2016), who also found sensitivity of *E. crypticus* to the increasing concentrations of As using mixtures of soil and sewage sludge. The Hormesis effect observed in the Oxisol is in agreement with results reported by González-Alcaraz and van Gestel (2016), who also observed a Hormesis effect on reproduction of *E. crypticus* in a series of dilutions of As-contaminated agricultural soils. According to these authors, the Hormesis effect could be related to compensation due to the interruption of the initial homeostasis and/or to a direct stimulation effect by As in the lowest concentrations. In reproduction tests with *E. andrei*, the observed toxicity agrees with previous studies that also found negative correlations between survival and reproduction of *E. andrei* and soil As contamination (Alves and Rietzler, 2015; Romero-Freire et al., 2015).

For Oxisol, both oligochaetes species were less sensitive than *F. candida*. On the other hand, in ATS, both oligochaetes were more sensitive than *F. candida*, with *E. crypticus* being always more sensitive than *E. andrei*. The slightly higher sensitivity of *E. andrei* in comparison to *F. candida* for As observed in ATS agrees with results obtained by Alves et al. (2016). These authors investigated the effect of sodium arsenate

411 in the reproduction of *E. andrei* and *F. candida* in ATS and in a Brazilian Oxisol and
412 estimated reproduction EC_{50} values of 22.7 (4.6–40.1) and 26.1 (14.2–37) mg As kg^{-1},
413 for *E. andrei* and *F. candida*, respectively, in ATS. For Oxisol, the EC_{50} reproduction
414 values for both species were higher than 135 mg kg^{-1} (the highest As concentration
415 tested). These data corroborate the relative slightly higher sensitive of *E. andrei*
416 compared with *F. candida*. In addition, the study of Alves et al. (2016) indicates that the
417 EC_{50} values estimated for ATS tend to be lower than those of Oxisol, which agrees with
418 the tendency observed in the present study. Despite that, in the present study, the
419 toxicity of As observed for *F. candida* in the Oxisol was higher than that found by
420 Alves et al. (2016) in an Oxisol (EC_{50} = 62.0 mg kg^{-1} in this study and EC_{50} > 135 mg
421 kg^{-1} by Alves et al., 2016). This difference can be attributed to a 30-day period observed
422 by Alves et al. (2016) to stabilize the As in the soil before starting the tests. Such period
423 might have promoted As adsorption to the soil particles and, consequently, lower
424 toxicity. In addition, it is known that As maximum adsorption capacity depends on
425 texture, mineralogy, pH and the presence of competitive ions, which might vary even
426 among soils from the same class (Campos et al., 2013a, 2013b, 2007, 2006).
427
428 4.1 Prevention value
429
430 Although it is known that the construction of SSD curves based on the
431 sensitivity of only four species constitute *per se* an analysis with low robustness, it was
432 performed to allow the estimation of HC_{50} and HC_5 values, because this was considered
433 decisive to discuss the adequacy of PVs established for As in soils of Minas Gerais
434 State, Brazil. Despite that, it should be noted that the HC values resulting from the
435 present study should be seen as preliminary values that must be complemented with
436 data on sensitivity of additional invertebrate species to As using the same soils (to
437 complement the present SSDs) or other soils representative of the same region (to apply
438 in other SSDs and generate additional HC values).
439 The PV value currently adopted for As (PV_{As}) by the State of Minas Gerais,
440 Brazil is 15 mg kg^{-1}. It was higher than the HC_5 values derived for ATS from the $EC_{20\text{-}total}$
441 values (Table 6). This means that the current PV_{As} established by law would affect
442 in 20% the reproduction of more than 5% of the species in a soil with arsenic
443 availability similar to ATS. On the other hand, the PV_{As} value in Oxisol probably would
444 affect in less than 20% much less than 5% of the invertebrate species.

HC_5 values based on $EC_{20\text{-total}}$ and $EC_{50\text{-total}}$ values in ATS constitute the most protective scenario considered in this study, since it aims to protect the reproduction of 95% of the test species in the test soil with the lowest As retention capacity (thus, the scenario with the highest As availability), and consequently, with the highest As toxicity. For all the other scenarios, the PV_{As} was, in general, equal or lower than the HC_5 and HC_{50} values estimated, and, because of that, PV_{As} can be considered adequate to preserve the soil habitat function since does not affect more than 50% the reproduction of more than 95% of the test species (CONAMA, 2009).

Additionally, it should be noted that the experiments performed in the current research were conducted without a soil incubation period, i.e., it was performed immediately after soil contamination. This fact caused a scenario of high As availability in each soil, but it is known that contaminants may suffer a phenomenon called “aging” in the soil that leads to the reduction of its availability/toxicity over time (Peijnenburg et al., 2012). Thus, the laboratory experiments performed might be overestimating the As availability/toxicity, when compared to soils from As-contaminated sites (Romero-Freire et al., 2015). Thus, prevention value for soils having similar physicochemical and mineralogical properties can be considered adequate, with an acceptable confidence level.

Furthermore, bioavailability can be considered a dynamic process (Lanno et al., 2004; Peijnenburg et al., 1999). Therefore, any environmental process that might alter contaminant availability should be considered during the applicability of the prevention value. In addition, soil attributes that might influence As availability and consequently toxicity levels, should also be considered a criterion to establish safety limits for environmental contaminants (Romero-Freire et al., 2015). This strengthens the necessity of establishing PV_{As} based on available concentrations.

The current prevention value can be very protective for soils that have large As adsorption capacities. On the other hand, it can be less protective for soils that have low adsorption capacity, in which available contents are close to total contents.

Variation of organism sensitivity observed in this research emphasizes the importance of selecting different organisms in ecotoxicological evaluations (Vašíčková et al., 2016). Besides considering that toxicity is variable among representative species regarding different exposure pathways to the contaminant, interactions between organisms in their ecosystem must be taken into consideration. In order to do that, it would be important to verify PV values within the ecosystem scenario context.

5. Conclusion

Ecotoxicological effects were more evident in ATS, followed by Oxisol and Inceptisol. *H. aculeifer* was the most resistant species to As in all tested soils, while *E. crypticus* and *F. candida* were the most sensitive species in ATS and Oxisol, respectively.

Results obtained in this research indicate that the As prevention value currently adopted by the Minas Gerais State, Brazil, is protective enough for the tested natural soils. The prevention value is higher than the HC_5 values derived for ATS both from $EC_{20\text{-total}}$ and $EC_{50\text{-total}}$ values and for Oxisol from $EC_{20\text{-total}}$ values. However, considering the remaining $HC_{5(total)}$ and $HC_{50(total)}$ values for the tested soils, the PV_{As} value was equal or smaller, and can be considered adequate for this tested organisms.

Additionally, results show that an evaluation of As PV adequacy must consider soil properties. Evaluation of As effects within an ecosystem context can provide additional data that allow the refinement of the PV_{As}.

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3 CONSIDERAÇÕES FINAIS

Os resultados deste trabalho de tese evidenciaram que os endpoints mais adequados para o estudo da fitotoxicidade do arsênio em plantas são a germinação inicial das plantas, área foliar e massa seca. Isso por que esses endpoints apresentaram-se sensíveis a contaminação por arsênio e os valores da concentração tóxica derivada através deles possuem menor intervalo de confiança.

A toxicidade do arsênio foi variável entre as espécies, tanto de plantas quanto organismos do solo. Mas, independentemente dos organismos testados, a toxicidade do arsênio foi mais evidente no solo artificial tropical, isso devido a sua menor capacidade em reter o arsênio.

Em relação ao atual valor de prevenção brasileiro, verificou-se que ele está dentro da faixa de valores que vem sendo utilizada por outros países como critérios de qualidade ambiental para arsênio nos solos. Contudo os ensaios ecotoxicológicos evidenciaram que esse valor é adequado para solos com elevada capacidade de adsorção de arsênio. Para os solos que apresentam baixa capacidade de adsorção será necessária a realização de ensaios adicionais para verificar com maior segurança os níveis de proteção do atual valor de prevenção para arsênio brasileiro.

4 ANEXOS

Table 1 Saline control of phytotoxicological tests. Mean value obtained between the treatments with sodium chloride used to simulate the same ionic strength of treatments 0, 8, 26, 84 and 270 mg of arsenic per kg of dry soil. No statistical differences ($p < 0.05$) were observed between control and saline treatments for any of the variables.

Soil	Endpoint	*O. sativa*	*Z. mays*	*S. bicolor*	*P. vulgaris*	*H. annuus*	*R. sativus*
ATS	EGC (%)	80.0 ± 1.53	93.3 ± 3.30	96.7 ± 3.30	-	100 ± 0.00	66.6 ± 6.00
	PH (cm/plant)	48.4 ± 1.21	53.4 ± 2.20	46.1 ± 0.85	-	40.8 ± 1.26	8.76 ± 0.11
	RLA (cm^2/plant)	18.6 ± 0.96	83.7 ± 3.70	59.6 ± 6.64	-	63.2 ± 3.44	35.6 ± 2.32
	SD (mm)	1.79 ± 0.07	5.88 ± 0.44	3.60 ± 0.12	-	3.40 ± 0.12	2.76 ± 0.08
	TG (%)	95.5 ± 2.22	100 ± 0.00	96.6 ± 3.33	-	100 ± 0.00	95.5 ± 2.22
	GSI	2.76 ± 0.12	2.99 ± 0.06	2.36 ± 0.08	-	1.70 ± 0.01	3.33 ± 0.13
	DM (g/plant)	0.13 ± 0.03	2.00 ± 0.21	0.27 ± 0.01	-	0.38 ± 0.00	0.13 ± 0.00
	CEL (leaves/plant)	4.00 ± 0.00	3.33 ± 0.33	5.00 ± 0.00	-	6.00 ± 0.00	4.80 ± 0.12
	PS (%)	83.6 ± 2.55	100 ± 0.00	100 ± 0.00	-	100 ± 0.00	97.6 ± 2.38
	SPAD	33.4 ± 1.31	25.0 ± 0.61	27.6 ± 0.08	-	32.7 ± 0.20	35.1 ± 0.66
	FnC (%)	80.0 ± 3.86	96.6 ± 3.30	96.7 ± 3.30	-	100 ± 0.00	95.3 ± 2.20
Oxisol	EGC (%)	82.0 ± 3.40	72.6 ± 6.50	51.3 ± 5.90	78.0 ± 2.80	80.9 ± 4.80	69.3 ± 3.20
	PH (cm/plant)	48.8 ± 0.55	55.8 ± 0.79	47.8 ± 1.32	37.4 ± 0.56	31.6 ± 0.53	13.8 ± 0.20
	RLA (cm^2/plant)	21.4 ± 0.74	86.6 ± 7.37	65.7 ± 5.54	198 ± 5.36	53.2 ± 2.60	54.9 ± 2.48
	SD (mm)	1.77 ± 0.02	4.82 ± 0.18	3.45 ± 0.06	3.27 ± 0.05	2.86 ± 0.04	3.12 ± 0.05
	TG (%)	95.5 ± 1.25	96.6 ± 2.10	90.6 ± 2.28	95.3 ± 1.65	98.0 ± 1.06	90.6 ± 2.33
	GSI	2.77 ± 0.05	2.47 ± 0.10	1.76 ± 0.08	1.84 ± 0.04	1.63 ± 0.05	3.21 ± 0.09
	DM (g/plant)	0.12 ± 0.00	1.39 ± 0.17	0.19 ± 0.01	0.78 ± 0.03	0.27 ± 0.00	0.19 ± 0.00
	CEL (leaves/plant)	4.00 ± 0.00	3.40 ± 0.16	5.00 ± 0.00	5.00 ± 0.00	5.97 ± 0.02	5.83 ± 0.09
	PS (%)	90.8 ± 2.33	99.3 ± 0.66	100 ± 0.00	97.9 ± 1.11	100 ± 0.00	98.4 ± 0.81
	SPAD	39.2 ± 0.24	30.5 ± 0.36	38.5 ± 0.53	28.9 ± 0.48	36.1 ± 0.38	38.1 ± 1.34
	FnC (%)	86.6 ± 2.13	95.3 ± 2.10	89.3 ± 2.30	92.0 ± 2.20	98.0 ± 1.00	90.0 ± 2.26
Inceptisol	EGC (%)	76.0 ± 5.80	94.6 ± 1.30	96.7 ± 1.30	69.3 ± 4.60	96.6 ± 1.20	76.0 ± 2.73
	PH (cm/plant)	50.2 ± 0.63	59.5 ± 0.78	53.8 ± 1.07	38.9 ± 1.14	35.0 ± 0.22	12.6 ± 0.21
	RLA (cm^2/plant)	20.2 ± 0.85	100 ± 5.10	64.3 ± 2.82	168 ± 6.17	60.8 ± 2.53	57.7 ± 3.08
	SD (mm)	1.73 ± 0.02	4.82 ± 0.17	3.38 ± 0.07	3.06 ± 0.05	2.88 ± 0.04	3.11 ± 0.10
	TG (%)	93.7 ± 1.38	98.6 ± 0.90	98.0 ± 1.07	94.0 ± 2.14	100 ± 0.00	94.2 ± 1.44
	GSI	2.69 ± 0.06	2.98 ± 0.03	2.36 ± 0.03	1.82 ± 0.06	2.01 ± 0.02	3.37 ± 0.05
	DM (g/plant)	0.12 ± 0.00	2.26 ± 0.36	0.24 ± 0.01	0.83 ± 0.03	0.30 ± 0.00	0.18 ± 0.00
	CEL (leaves/plant)	4.00 ± 0.00	3.80 ± 0.10	5.00 ± 0.00	5.00 ± 0.00	6.02 ± 0.02	5.89 ± 0.07
	PS (%)	91.1 ± 2.53	98.6 ± 0.90	98.6 ± 0.91	98.5 ± 0.96	98.6 ± 0.90	97.5 ± 0.93
	SPAD	39.7 ± 0.56	33.7 ± 0.36	37.5 ± 0.40	31.8 ± 0.64	36.1 ± 0.38	44.4 ± 0.98
	FnC (%)	85.3 ± 2.33	97.3 ± 1.10	97.3 ± 1.20	92.7 ± 2.30	99.3 ± 0.60	93.3 ± 1.53

Figure 1 Reduction in biomass (%) of *Eisenia andrei* when exposed to gradients of increasing arsenic concentrations in an artificial tropical soil, an Oxisol and an Inceptisol. * - treatments that significantly differ from control by Dunnett's post hoc test ($p \leq 0.05$).

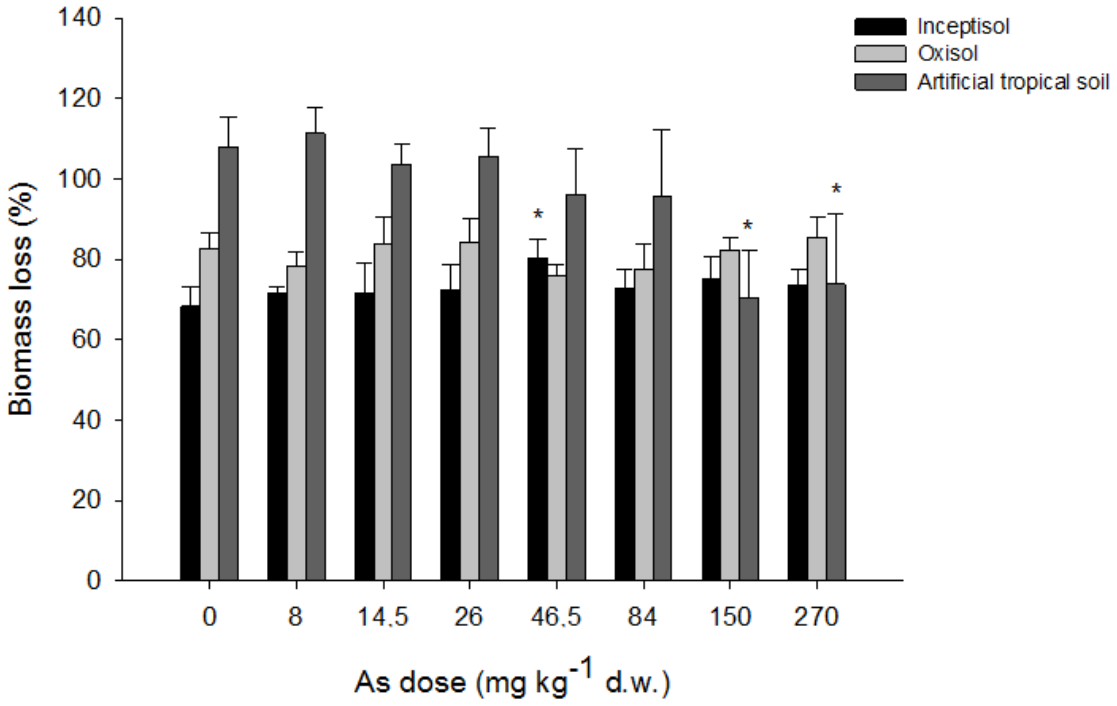

Figure 2 Sorghum plants (*Sorghum bicolor*) grown in artificial tropical soil (S. Artificial), Oxisol (Latossolo) and Inceptisol (Cambissolo) artificially contaminated by arsenic (0, 8, 14.5, 26, 46.5, 84, 150, 270 mg kg^{-1}).

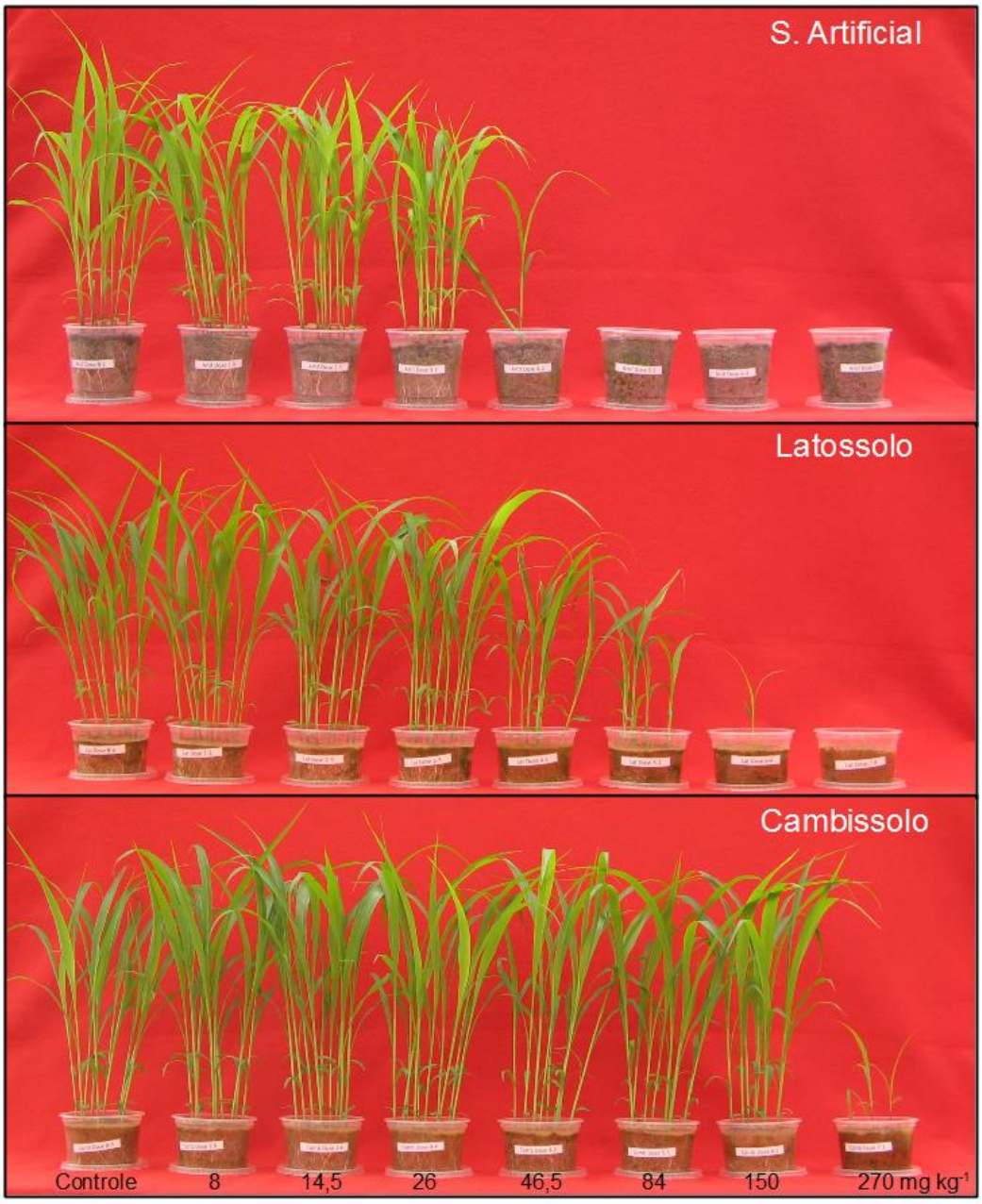

Figure 3 Radish plants (*Raphanus sativus*) grown in artificial tropical soil (S. Artificial), Oxisol (Latossolo) and Inceptisol (Cambissolo) artificially contaminated by arsenic (0, 8, 14.5, 26, 46.5, 84, 150, 270 mg kg^{-1}).

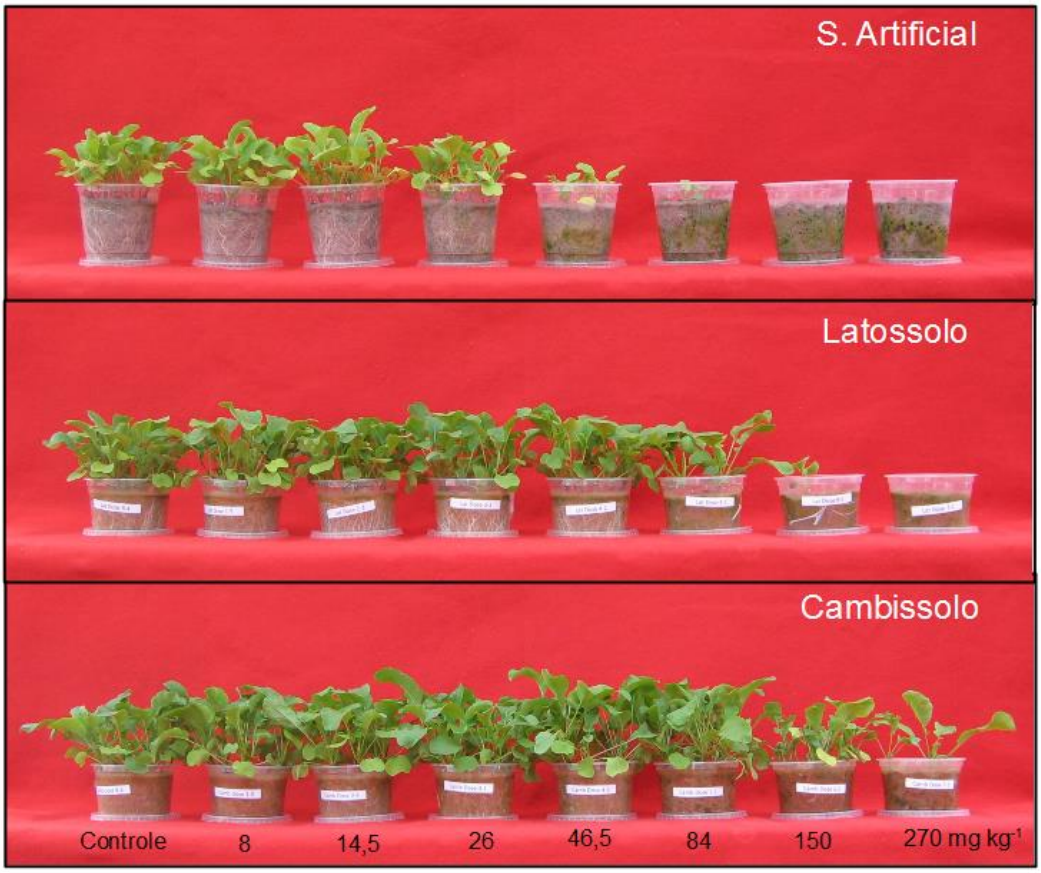

Figure 4 Maize plants (*Zea mays*) grown in artificial tropical soil (S. Artificial), Oxisol (Latossolo) and Inceptisol (Cambissolo) artificially contaminated by arsenic (0, 8, 14.5, 26, 46.5, 84, 150, 270 mg kg^{-1}).

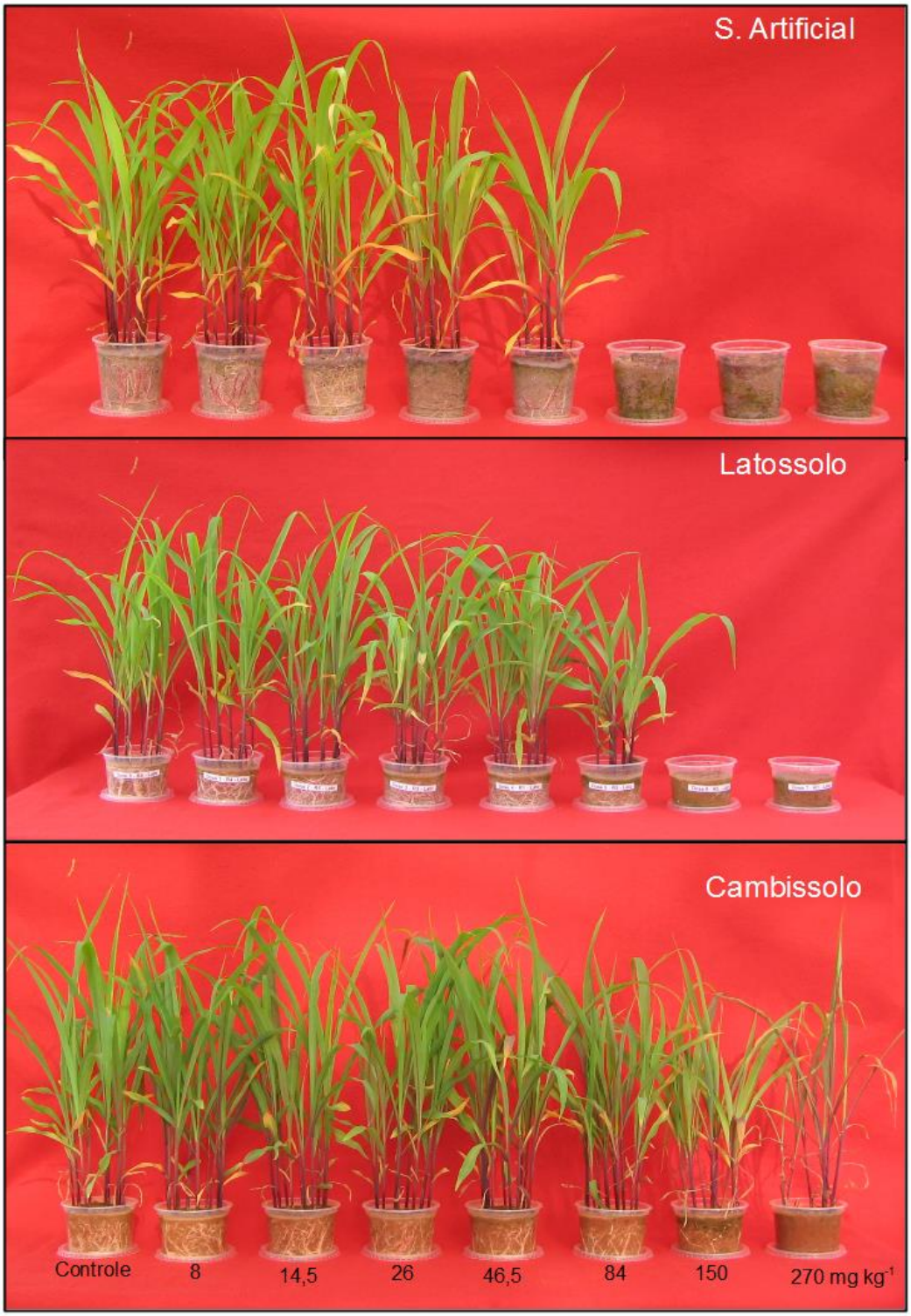

Figure 5 Sunflower plants (*Helianthus annuus*) grown in artificial tropical soil (S. Artificial), Oxisol (Latossolo) and Inceptisol (Cambissolo) artificially contaminated by arsenic (0, 8, 14.5, 26, 46.5, 84, 150, 270 mg kg^{-1}).

Figure 6 Bean plants (*Phaseolus vulgaris*) grown in artificial tropical soil (S. Artificial), Oxisol (Latossolo) and Inceptisol (Cambissolo) artificially contaminated by arsenic (0, 8, 14.5, 26, 46.5, 84, 150, 270 mg kg^{-1}).

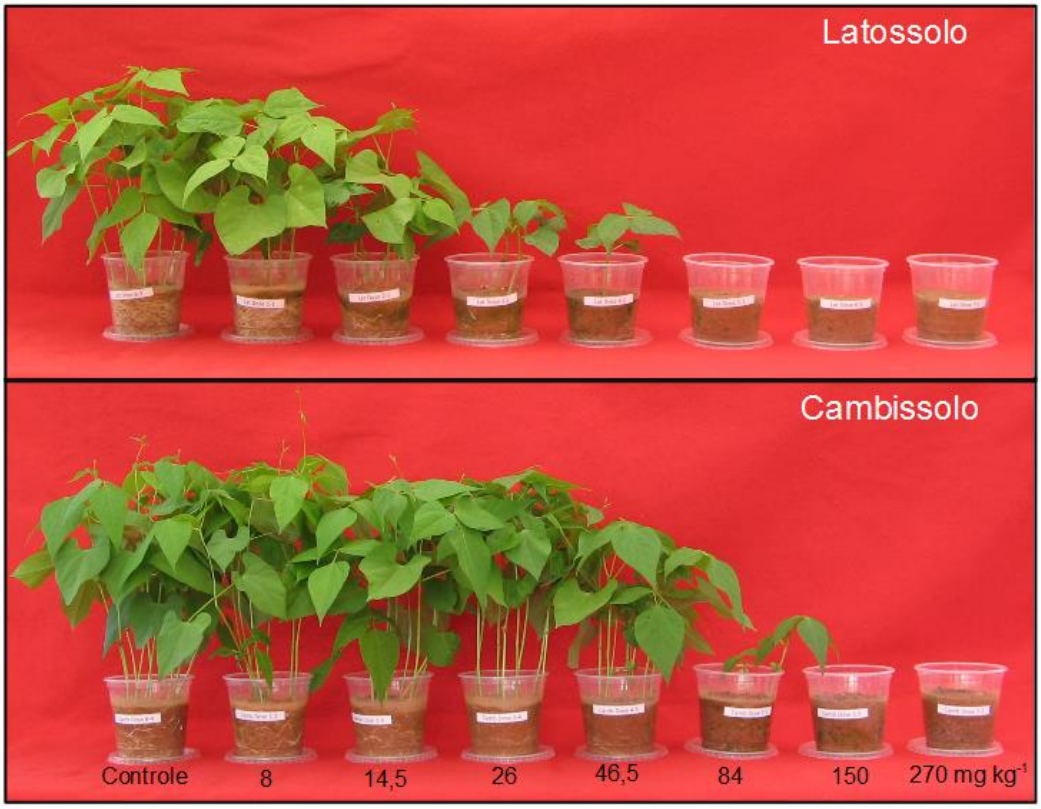

Figure 7 Rice plants (*Oryza sativa*) grown in artificial tropical soil (S. Artificial), Oxisol (Latossolo) and Inceptisol (Cambissolo) artificially contaminated by arsenic (0, 8, 14.5, 26, 46.5, 84, 150, 270 mg kg^{-1}).

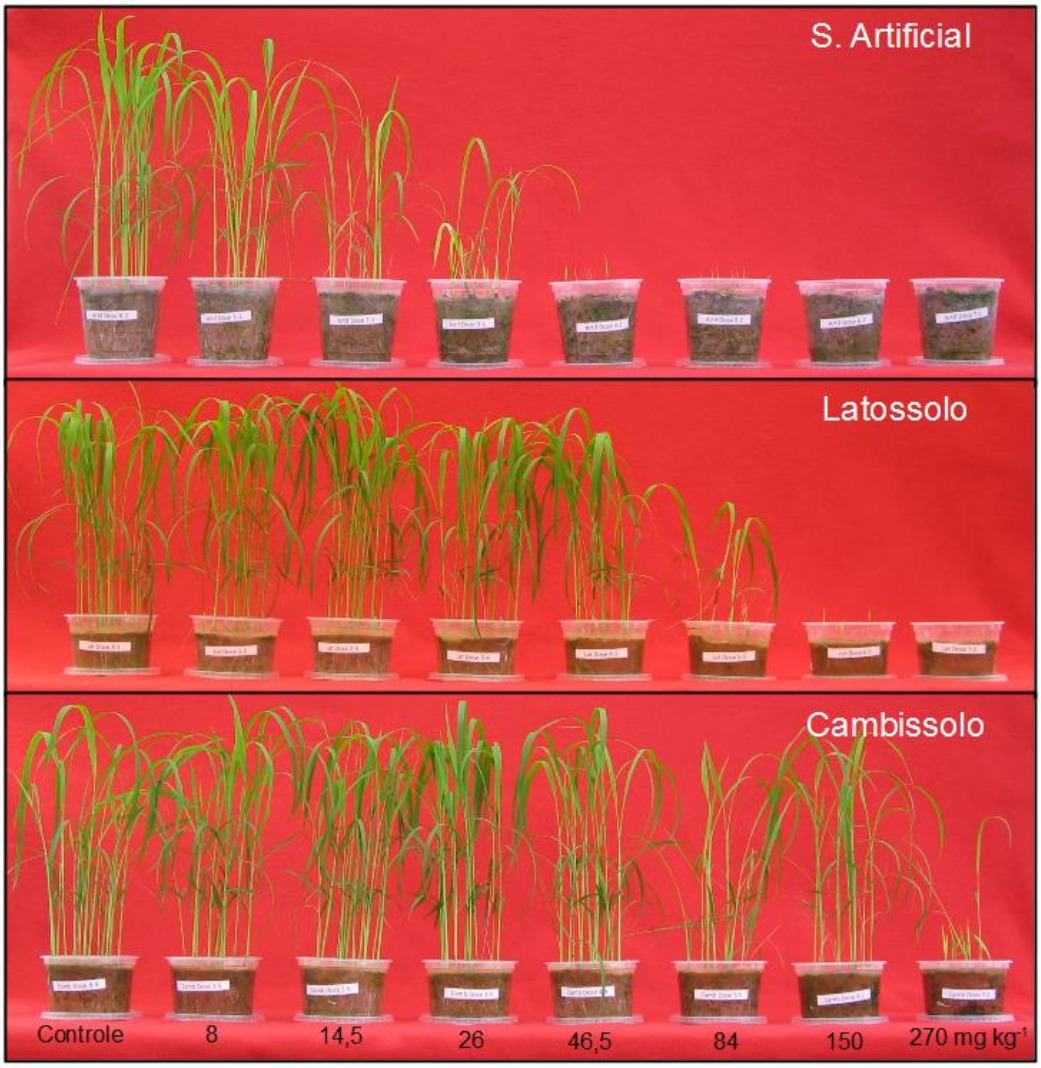

Table 2 Mean number of surviving and juvenile of mites, collembolans, enchytraeids and earthworms in saline controls. Treatments performed with sodium chloride to simulate the same ionic strength of treatments 0, 150 and 270 mg of arsenic per kg of dry soil. No statistical differences ($p < 0.05$) were observed between control and saline treatments for any of the variables.

	Surviving			Juvenile		
Dose	ATS	Oxisol	Inceptisol	ATS	Oxisol	Inceptisol
			Mites			
Saline 0	10.0 ± 0.00	9.55 ± 0.17	9.00 ± 0.37	221 ± 9.57	239 ± 11.5	257 ± 6.46
Saline 150	10.0 ± 0.00	9.40 ± 0.24	8.80 ± 0.37	251 ± 38.1	218 ± 12.1	222 ± 17.9
Saline 270	9.66 ± 0.33	9.00 ± 0.31	9.40 ± 0.40	263 ± 28.7	199 ± 6.16	264 ± 26.3
ANOVA	-	0.259	0.613	0.274	0.095	0.209
			Collembolans			
Saline 0	10.0 ± 0.00	9.75 ± 0.25	9.80 ± 0.20	1104 ± 80.9	656 ± 85.1	899 ± 45.5
Saline 150	9.6 ± 0.24	9.20 ± 0.37	9.25 ± 0.48	1159 ± 87.5	471 ± 59.9	747 ± 16.9
Saline 270	10.0 ± 0.00	9.40 ± 0.40	9.80 ± 0.20	1058 ± 159	445 ± 73.9	921 ± 64.3
ANOVA	-	0.590	0.369	0.812	0.143	0.075
			Enchytraeids			
Saline 0	-	-	-	2065 ± 144	625 ± 151	239 ± 44.3
Saline 150	-	-	-	1606 ± 70.2	952 ± 30.1	309 ± 94.5
Saline 270	-	-	-	1685 ± 244	744 ± 66.7	469 ± 58.5
ANOVA	-	-	-	0.141	0.099	0.060
			Earthworms			
Saline 0	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	68.5 ± 6.13	14.6 ± 2.40	34.5 ± 4.55
Saline 150	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	76.7 ± 5.13	19.7 ± 4.60	36.2 ± 3.35
Saline 270	10.0 ± 0.00	10.0 ± 0.00	10.0 ± 0.00	54.5 ± 6.06	12.3 ± 0.33	33.7 ± 3.88
ANOVA	-	-	-	0.065	0.349	0.901

Figure 8 Average reproduction (A, ± standard deviation) and survival (B, ± standard deviation) of *Hypoaspis aculeifer* when exposed to an Oxisol, an Inceptisol and an artificial tropical soil (ATS) artificially contaminated with increasing concentrations of available arsenic.

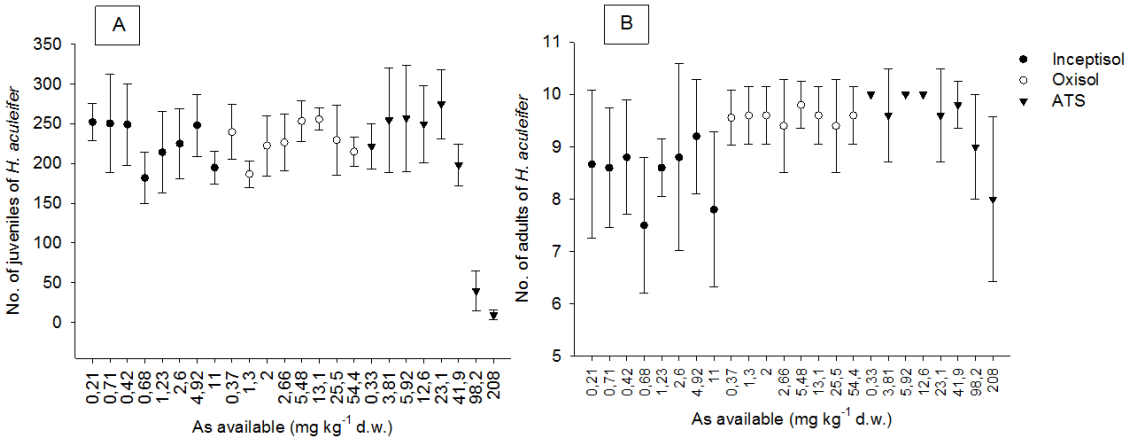

Figure 9 Average reproduction (A, ± standard deviation) and survival (B, ± standard deviation) of *Folsomia candida* when exposed to an Oxisol, an Inceptisol and an artificial tropical soil (ATS) artificially contaminated with increasing concentrations of available arsenic.

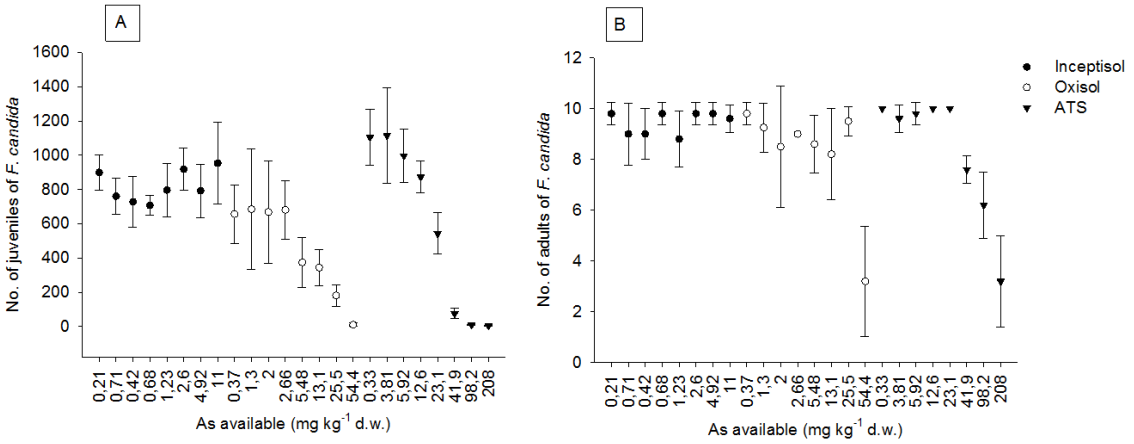

Figure 10 Average reproduction (A, ± standard deviation) and survival (B, ± standard deviation) of *Eisenia andrei* when exposed to an Oxisol, an Inceptisol and an artificial tropical soil (ATS) artificially contaminated with increasing concentrations of available arsenic.

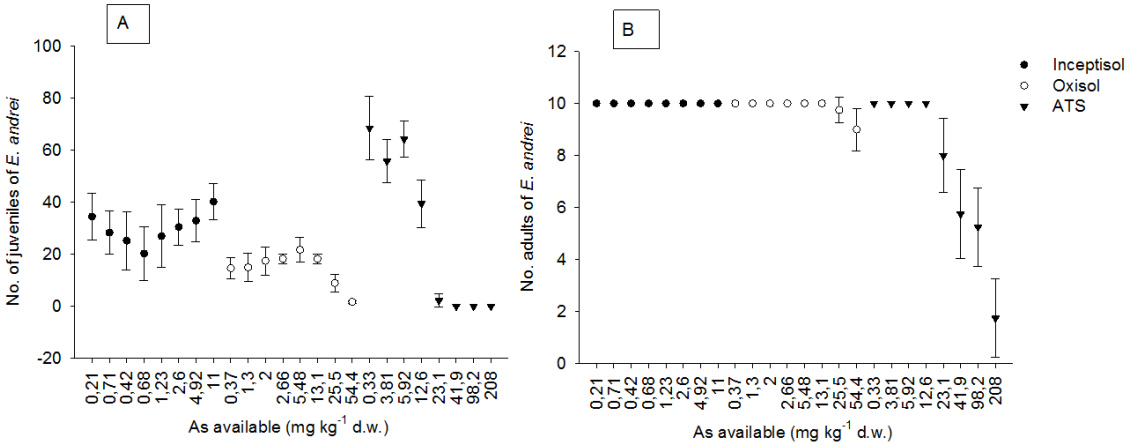

Figure 11 Average reproduction of *Enchytraeus crypticus* when exposed to an Oxisol, an Inceptisol and an artificial tropical soil (ATS) artificially contaminated with increasing concentrations of available arsenic.